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REFLECTIONS OF A MEDICAL PARASITOLOGIST*

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INTRODUCTION

Parasitology may be broadly defined as the sum of knowledge of those organisms, both animals and plants, which live on or in other organisms, obtaining nourishment and protection from the latter without compensation. In this sense the field of parasitology includes bacteriology, virology and mycology, as well as protozoology, helminthology and entomology. The more usual definition limits the field to the area of animal parasites. It is this more restricted concept about which I am concerned in my Presidential Address before the American Society of Parasitologists.

By interest and training a parasitologist may be primarily or exclusively a student of the morphology, physiology, ecology, taxonomy or phylogeny of animal forms in their relationship to one another. In other words, he may be a parasitologist in the broad sense of the term. A very considerable amount of valuable fundamental biological knowledge has developed through the years as a result of investigations of this nature. On the other hand, by interest and training, or by circumstance and necessity, a parasitologist may have directed his attention to an applied aspect of the subject, so that his contributions are related primarily to the field of economic entomology, veterinary science or human medicine. In these areas parasitologists have also made many noteworthy contributions to human knowledge.

QUALIFICATIONS OF A MEDICAL PARASITOLOGIST

In applied parasitology a properly qualified individual will have had several years of background training in the biological sciences, and particularly in zoology. Moreover, he will have had several years of graduate study in comparative parasitology, with special investigation in one or more problems. Medical parasitology is essentially a postgraduate discipline, acquired by years of experience in the study and evaluation of the problems of human parasitology. Not only is the medical parasitologist concerned with the etiology, epidemiology, diagnosis and control of parasitic diseases of man, but to an equal degree with the relationship of the etiologic agent to the tissues of the human body, the host's reactions provoked by the parasite and the signs and symptoms evoked by the infection. This requires a study of patients harboring the parasites, both individual patients and population groups which present problems in public health.

* Address of the retiring president of the American Society of Parasitologists, December 8, 1948, New Orleans, Louisiana.

While a medical degree acquired subsequent to graduate training in parasitology provides an ideal combination of qualifications, very few medical parasitologists in the United States have been able to undertake the necessary formal training. Although combined doctorates in natural science and medicine are frequently conferred in European universities, the speaker does not consider this to be a practical program within the foreseeable future in the United States. Lacking the discipline of clinical medicine the parasitologist must not expect clinicians to accept him as a colleague until he has demonstrated a comprehension of parasitic infections from the point of view of the physician, until he has an appreciation of medical ethics and cooperates with, rather than attempts to dictate to the physician.

Physicians are in need of the special skill and training of well-qualified medical parasitologists. As a team the physician and the parasitologist can make remarkable progress, and serious errors which might be perpetrated by either one alone will be avoided or minimized. There is a tendency for younger parasitologists gaining experience in the medical aspects of their subject to be disturbed if they are not immediately recognized as equals by the clinician. While an occasional physician may be antagonistic, this is not a frequent occurrence, provided the parasitologist does not overstep his prerogatives. It would be helpful for a well-trained parasitologist, when he thinks he has been slighted or his opinion has been disregarded, to examine his own attitude and to discover if he has not been primarily responsible for the alleged affront. In the speaker's long contact with physicians he has rarely encountered anything but sympathetic cooperation and appreciation for the assistance he had rendered.

One of the more difficult problems which may be of concern to a medical parasitologist who lacks a medical degree is that of handling questions concerning the clinical aspects of his subject. If the inquirer is a physician and, knowing that the parasitologist is not a physician, requests the parasitologist's opinion, there is no reason why factual information should not be given, with the understanding that the parasitologist will not recommend a clinical procedure which would or might endanger a patient's health and thus harm the reputation of the physician. The parasitologist can not assume the legal responsibility of directing the management of the patient. Since he does not have the legal right to do so, he must not advise the physician to carry out radical measures merely to test theories or experimental data. If the inquiry comes from a layman and has clinical implications, it is necessary to ask the individual to have his physician make the request. An exception to this rule is that in which information on the methods of acquiring and controlling certain diseases may be explained in non-clinical terms, thus providing a service which will be of educational value to the individual and his community. If the inquiry comes from a newspaper representative a most serious problem is faced. The least that can be done is to require an accurate typescript for examination and correction. Preferably this should be approved by the institution's administrative officers. Even then a conservative statement out of its context may be featured in print as a "punch line" or as a sensational discovery. This type of publicity is harmful to the individual and to the profession.

The next question which presents itself is with reference to a young physician acquiring special training in parasitology. Such training is highly desirable and

one or more years of specialization in medical parasitology will provide valuable technical experience. Nevertheless, without an adequate background in zoology the situation is much like that of a student of vital statistics who failed to take basic courses in mathematics during his college course: there will always be a feeling of uncertainty about fundamentals. With the practical applications of parasitology so clearly in the foreground, it is difficult for the average physician without special training to appreciate the importance of comparative parasitology, of the relationship of parasite groups to free-living animals, of taxonomy and parasitological nomenclature, all of which the trained parasitologist appreciates. Had the physician acquired this knowledge before specializing in clinical medicine, both the background knowledge and subsequent training in medicine would be viewed in their proper perspective. Furthermore, if a physician is to become specialized in any group of infectious diseases, his medical training in bacteriology is more likely to provide an area of interest than is medical parasitology, with which as a medical student he probably had very little opportunity to develop an acquaintance or interest.

The speaker's own training and experiences may be of general interest and usefulness in illustrating the views which have thus far been expressed. With high-school subjects primarily in English, Latin and history and college specialization in botany and zoology, an opportunity was offered for graduate study in a large mid-western university and at the same time to serve as research assistant to the distinguished dean of American parasitologists. Requirements for majoring in parasitology did not prevent, but rather encouraged broad graduate training in other fields of biology, including experimental zoology, ecology, entomology, comparative anatomy, mycology and physiological botany, and, in addition, biochemistry and pathology. Upon obtaining the master's degree and doctorate and after two additional years as instructor in the same university, the speaker was offered the position of parasitologist in the newly opened Peking Union Medical College, Peking, China. During a period of eight years unusual opportunities were provided for gaining a wealth of practical experience in medical parasitology in the clinic, the hospital and in the field. Thereafter a chair in parasitology was created in the School of Medicine, Tulane University of Louisiana and for more than twenty years this institution has been the center of the speaker's activities.

Although it is highly improbable that any other parasitologist will have training and opportunities exactly comparable to those which have just been described, many individuals have had as good an academic background and have had experience with human parasitic diseases in highly endemic regions of the world. Thus, since a community of interest exists, the speaker may possibly be pardoned for drawing on his own experience again in outlining the contributions which he believes a medical parasitologist should make to his own profession and to the broad field of human welfare.

FUNCTIONS OF THE MEDICAL PARASITOLOGIST

First of all the medical parasitologist must be a *teacher*. As in so many other fields of human experience, the words of Chaucer's Canterbury pilgrim are applicable here: "Gladly would he learn, and gladly teach." He should be kindled with enthusiasm in the knowledge that his subject is a vital and integral part of the medical sciences. Yet he should be conscious of his own limitations and should refer to

his personal experiences with caution and humility, since his interpretations may have been based on inadequate observation or even on prejudice.

The medical parasitologist should have a comprehensive knowledge of animal parasitology in its relation to human disease and should be able to present the essential facts to medical students, post-graduate physicians, graduate students and laymen, in language which each group can properly interpret. Appreciating that parasitic diseases constitute a very large group of the ills of mankind, he will realize that their importance varies in different regions of the world. He will know his geography and the regions where each major parasitic infection is particularly significant in clinical medicine and public health. He will understand the pathogenesis of each infection, will be readily conversant with the most useful therapeutic procedures for treatment of patients suffering from parasitic disease and will keep well informed on the modern methods of control, imparting this information in the proper form and at the proper time.

Presentation of the essentials of medical parasitology and parasitic diseases varies depending on the level of training and experience of the audience. If the basic course in parasitology in a medical school is taught in conjunction with bacteriology or preventive medicine in the first or second medical year preceding the basic course in pathology, the subject matter is necessarily limited primarily to a consideration of the parasites and their epidemiology. If the student already has taken basic work in pathology, the subject matter will include parasite-host interrelationships and tissue damage. If the student has entered on clinical work (and this is the place where the speaker is convinced medical parasitology should be taught), then the parasitologist has an opportunity to present his subject in its full perspective. In formal classes, review courses or occasional lectures for the physician, the material must be adapted to the post-graduate level. Here there must be a minimum of technical detail concerning the parasite and an elaboration of the clinical aspects of parasitic diseases. For students working towards graduate degrees in medical parasitology considerable emphasis on comparative parasitology is required, but examples and project studies in the medical field are justified, otherwise the graduate student should pursue his training in a university's department of zoology. Finally, lectures or radio addresses intended primarily for a lay-audience must be presented in clear, simple language, essentially free of technical terminology, although approximately ten per cent of new ideas containing semi-technical information will be well received and will serve as a distinct stimulus to the listeners in obtaining additional education in the epidemiology and prevention of parasitic diseases.

Visual aids should be employed to supplement rather than replace his lectures and conferences. Good blackboard diagrams, freshly prepared, are frequently more effective than are movie films in illustrating the subject. His material should be carefully worked up in advance, clearly outlined in his own mind and presented slowly enought and distinctly enough so that each person in the audience will follow his thought. Formal lectures should serve only to outline the material and to develop an initial interest. Much more valuable teaching results from personal contact with the student in conferences and particularly in the laboratory where practical experience and proficiency are developed by the student. Each laboratory period should have at least one demonstration of living parasites which are harmful to man. Today

it is relatively easy to provide the student with living material, including cultures of parasitic amebae, *Trichomonas, Balantidium,* species of trypanosomes and leishmanias, likewise embryonated eggs of *Ascaris* and *Enterobius,* larvae of hookworm and *Strongyloides,* living microfilariae, muscle tissue of rats infected with *Trichinella,* and snails discharging one of more species of cercariae.

The medical parasitologist must be an experienced *laboratory diagnostician.* He should have close contact with, or be an integral part of, the diagnostic service of the clinics or hospitals served by his medical institution, so that coprological and blood specimens will be routinely referred to him for examination. He should provide an intelligent interpretation of his findings and may suggest, but should not attempt to dictate, how the clinician will handle the patient. Moreover, these services should provide good diagnostic material for use in the teaching program. As the experience of the medical parasitologist becomes generally recognized, unusual and more involved diagnostic problems will be referred to him. Frequently these diagnoses will not be difficult; at times specific diagnosis can not be made from examination of the available material. Again, the material referred for confirmation may contain very obvious artefacts, as cotton fibers which have been confused with microfilariae in a blood film, plant hairs mistaken for *Strongyloides* larvae, or parenchyma cells of partly digested vegetables misdiagnosed as helminth's eggs. Occasionally the diagnostic specimen will provide a very interesting problem which will warrant careful investigation.

The admittedly most difficult diagnostic problem in the entire field of parasitology is that of *Endamoeba histolytica.* Because of the present-day consciousness of the potential danger of amebiasis on the part of physician and layman alike, unconfirmed positive diagnoses are beginning to flood hospitals and private clinics, to the detriment of the medical profession. The medical parasitologist can help to alter this embarrassing situation by tactful cooperation .

The medical parasitologist should be an active *contributor to the field of human knowledge.* His area of interests provides a vast wealth of subjects for investigation. The speaker has consistently stated that investigation in medical parasitology should provide opportunities for fundamental discoveries in biology as well as solutions to practical problems.

Many parasitologists, like their colleagues in other sciences, become discouraged because of heavy routine duties and because financial assistance does not immediately become available for research. These are serious obstacles which must be surmounted. The person who has scientific curiosity will always find ways of satisfying his desire to solve some of the problems which he encounters; and he will frequently inspire younger men and women with his zeal for new information.

The natural development of medical parasitology as a field for investigation has paralleled that of comparative parasitology. From a description of new species parasitizing man and their classification, through life cycles, the trend has been towards the physiology of the parasite and its relation to its environment. In medical parasitology special emphasis is placed on the natural history of the parasite both in its extrinsic environment and in the human host, thus, on the one hand, providing a basis for epidemiology and control and, on the other hand, the pathogenesis of the infection, which, in turn, serves as a clue for interpreting the clinical findings and the effects of chemotherapeutics.

The medical parasitologist should be *skilled in writing* both technical and relatively non-technical papers. He must present his ideas clearly, concisely and in good idiom. Here, again, he should keep his audience in mind. When he is writing for publication in a professional journal which is read primarily by parasitologists, he will present his results in the technical language of his field; when he writes for a clinical journal the etiologic agent is fitted into its clinical setting. When his publication is intended for a more general audience his style and language must be similarly adapted. The material presented should be stimulating but written as objectively as possible, without controversy or vitriolic criticism. As a scientist the writer will indicate that his views or conclusions are limited to the observations presented or referred to in the body of his paper.

The medical parasitologist should be an *active member of* the more important *scientific societies* in his field and should loyally support their activities. He should regularly attend the meetings of one or more of these societies and contribute to their scientific programs.

The medical parasitologist should *participate in the activities of his community.* He should be interested in the public schools of his city and its social welfare organizations. As a taxpayer he should understand its political economy and should utilize his privilege of suffrage in local and national elections. He should be identified with some institution which encourages inquiry into religion. In other words, as a citizen he should not be an unknown social organism who asks only to be left alone to pursue his professional activities.

Finally, the medical parasitologist should *have a hobby,* or at least a diversion from his routine duties. It may be hunting or fishing, gardening or raising chickens, tinkering in a workshop, painting, muscial appreciation, or any other interest which regularly divorces him for a time from his professional routine.

While certain medical parasitologists have an opportunity to enter into the several aspects of their professional fields, others are of necessity confined primarily to teaching or to investigation. Teaching without research eventually causes a loss of personal warmth for the subject matter. Research without opportunity of contributing to the formal instruction of others frequently narrows the perspective and produces an unsocial attitude. When a distinguished American scholar was once asked whether he preferred teaching or research, he promptly replied: "I choose both." This might well be the aim of each one of us. Moreover, in employing his professional talents to best advantage the medical parasitologist should have as his over-all objective the betterment of human beings and the world in which they live.

This address would not be complete unless some comments were made with reference to present-day and future opportunities in the United States in the field of medical parasitology. It has been emphasized that this field requires special training and experience. Furthermore, it has been shown that there is a distinct need for the medical parasitologist, that very few individuals have been able to take the medical degree subsequent to specialization in parasitology and that very few physicians have qualified as experts in the subject. There are and will continue to be openings in medical schools for experienced medical parasitologists in departments of microbiology, preventive medicine and public health, as well as in departments of medicine. Similarly, there will be openings in both Federal and State health agencies

and in the Armed Services. Certain of the positions will be open only to individuals with medical degrees, since they carry responsibilities for which only a licensed physician can qualify. It may be hoped that a position in parasitic and tropical diseases will not be filled by the appointment of an individual just because he has an M.D. degree, but only if he is really qualified for the position. In the future young men who are considering a career in medical parasitology might well plan their course work in college with the double objective of science and medical doctorates. Without such planning certain prerequisites for one or the other are apt to be early by-passed and valuable time will be lost later on in fulfilling these requirements. Nevertheless, the speaker is certain that there will be adequate openings in medical parasitology for those who have demonstrated their professional ability and fitness to work in a medical group. Such individuals may have had their graduate training in an academic atmosphere but they can obtain necessary orientation and experience only in a medical environment.

This is my credo and my message to my colleagues in the American Society of Parasitologists who have honored me with the Presidency of the Society for 1948.

SCREENING LARGE NUMBERS OF NEW CHEMICAL COMPOUNDS FOR ANTHELMINTIC ACTIVITY USING INFECTIONS WITH *NIPPOSTRONGYLUS MURIS* IN MICE

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Anthelmintics in use today, or those which have only recently been supplanted (Findlay, 1939), were known in ancient medicine (santonin, aspidium, chenopodium) or arose empirically in somewhat more recent general practice (thymol, chloroform, β-naphthol) or resulted from special studies of compounds known to be active in other respects or to be related to biologically active compounds. Thus, carbon tetrachloride (Hall, 1921) and tetrachlorethylene (Hall and Shillinger, 1925) were studied in worm infections of dogs because of their similarity to chloroform. Following the demonstration of the antibacterial properties of hexylresorcinol (Leonard, 1924), this compound and related forms were studied as potential ascaricides by Lamson and his colleagues (Lamson & Brown, 1936). Phenothiazine was selected for trials in worm infections of livestock because of its previously demonstrated activity as an insecticide and its availability (Harwood, Jerstad and Swanson, 1938).

Another approach to the search for new and better anthelmintics is to test large numbers of compounds, more or less at random, as illustrated by the quest for new antifilarial drugs (Hewitt, et al, 1947), which has thus far led to the discovery of Hetrazan (1-carbethoxy-4-methylpiperazine) and the investigations of collaborators in the schistosomiasis program of the Office of Scientific Research and Development (Shubert, 1948) using infections with *Schistosoma mansoni* in mice.

We are attempting to develop an *in vivo* method suitable for "screening" large numbers of chemical compounds for possible activity against intestinal helminth infections and this paper is a report of the early phases of our experience with infections of mice with *Nippostrongylus muris* (Yokagawa, 1920). This particular host-parasite combination was chosen because it seemed to meet best the requirements which we considered as most important, namely: (1) standardized experimental infections must be easily produced in large numbers of test animals, (2) the host must be small in order to conserve drug which is frequently available in quantities of only a few grams in the case of new compounds, (3) the host must be available as a uniform strain in large numbers at low cost and easy to care for and dose, (4) the evidence of drug activity must be easy to read, and (5) the results must be capable of reproduction. Other infections which were considered but discarded, at least temporarily, were: (I) *Trichinella spiralis* in mice, (II) *Strongyloides ratti* in rats, (III) *Syphacia obvelata* and (IV) *Aspicularis tetraptera* in mice, (V) *Trichostrongylus colubriformis* in rabbits, (VI) *Ascaridia galli* and (VII) *Heterakis gallinae* in chickens, (VIII) *Hymenolepis nana* in mice, (IX) *Raillientina cesticillus* and (X) *Choanataenia infundibulum* in chickens. These infections were not used for routine screening because they cannot be transmitted readily experimentally

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(III, IV); require too large a host (V); offer difficulties in obtaining quantitative counts of adults (I, II) in large-scale experiments; or are not representative of the most important problem group which is considered to be the nematodes (VIII).

Unquestionably the most important criticism of the use of *N. muris* is that it serves only as a substitute for parasites of economic importance and past experience has shown that drugs most characteristically are rather specific in action. However, since the most desirable anthelmintic would be one active against many kinds of worms, the use of almost any species for screening purposes may lead to such a discovery, although, admittedly, some useful drugs may be overlooked. At any event it seemed worth testing several thousand compounds against *N. muris* infections to serve as a background for determining the value of the test.

Materials and Methods

Details of the life cycle and development (Yokagawa, 1922; Schwartz and Alicata, 1934), activity of the larvae (Africa, 1931a), immunity and host-parasite relationships (Africa, 1931b; Chandler, 1937; Graham, 1934; and Porter, 1935) of *N. muris* are well known. Only the modifications in techniques developed to serve our particular needs will be discussed here.

The strain of *N. muris* was received from the Parasitology Department, School of Hygiene and Public Health, The Johns Hopkins University, in August 1946, in infected rats and has since been maintained in these Laboratories by passage at 21-day intervals in young rats. Several sub-strains were maintained simultaneously so that cultures could be made each week from feces collected between the 10th and 14th day of an infection. Egg-bearing feces were mixed with granular bone charcoal for cultivation. Larvae for infecting were isolated by means of the standard Baermann technique. The larvae were counted by suitable dilution techniques and concentrated by sedimentation.

The concentrated larvae were resuspended in 0.10% agar solution so that the desired number of larvae to be injected into each animal was contained in 0.1 cc of fluid. An agar solution of this concentration was found, by empirical means, to have the correct viscosity to prevent the rapid settling of the larvae and its use eliminated the necessity for constant shaking of the inoculum to insure uniform distribution of the larvae. The agar had no deleterious effect on either the larvae or the host.

It was found that larvae frequently become stuck in old needles or needles smaller than 22 gauge, therefore new needles no smaller than 22 gauge were regularly used for inoculation. A 0.25 cc tuberculin syringe was used and only the 0.1 cc dose drawn up each time. This is important when using agar as a suspending medium because peculiar currents probably due to the viscosity of the fluid which develop in larger syringes used for multiple doses concentrate the larvae in the last dose delivered. The coefficient of variation of the number of larvae dispelled in each 0.1 cc inoculum was found to be about 10% for an inoculum of 500 larvae.

Hosts were infected by intraperitoneal injection of larvae since this is the easiest method to use in infecting large numbers of individuals at one time and since limited studies failed to show that other methods gave particularly better results.

Young mice of about 4 weeks of age and weighing 10 to 12 g. were used for all routine testing. All mice were obtained from a single source (Vanderwerken-Stam-

ford, Conn.) and none has ever shown evidence of natural infection with *N. muris*. Porter (1935) in comparing infections with *N. muris* in rats and mice concluded "that the mouse is a somewhat abnormal host. This was demonstrated by the longer prepatent period, smaller percentage development, lower egg production and shorter duration of infestations in mice than in rats." However, Porter's comparisons were made between mice and rats of the same chronological age and since mice develop more rapidly than rats, they were closer to maturity and thus presumably might exhibit a greater resistance due to their age. If some of Porter's data are rearranged to compare infections in animals more nearly comparable in respect to physiological age, i.e., younger mice with older rats, the differences between the infections in the two species are less striking. At any rat, Porter's data indicate that good infections may be obtained in young mice and our own experience (Table 1) bears this out. While the variation in the number of worms established in a

TABLE 1.—*The number of adult* N. muris *established in the intestines of individual mice (10–14 g. weight) in ten separate tests eight days after intraperitoneal inoculation of approximately 400–500 larvae*

Test Number	1	2	3	4	5	6	7	8	9	10
Approximate no. larvae injected	400	500	500	500	500	500	400	480	450	500
No. of worms established	98	245	173	229	87	102	83	161	97	82
	51	238	128	174	103	82	161	158	199	102
	305	160	216	111	123	132	37	216	61	43
	20	72	236	168	149	98	65	165	162	177
	86	163	124	207	95	61	61	139	125	60
	97	278	184	179	77	47	40	133	147	56
	26	121		81	58	134	185	137	88	234
	147	234		163	208	91	62	69	186	132
	87	117		172		89		227		33
				94		137				179
				42						
				6						
				73						
Mean no. of worms established	102	181	177	131	112	97	87	156	133	110
Approximate % development	25	36	35	26	22	19	22	33	30	22

group of mice may be quite large, the average number of worms in a control group is great enough to permit the effect of active drugs to be readily noticed.

In mice of the age and size used routinely, more than 20% of the larvae inoculated usually developed to maturity in the intestine. Approximately five hundred larvae were injected as a standard inoculum since this gave the maximum number of adult worms in the intestine without causing undue damage during the migration of the larvae through the lungs.

The endpoint or evidence of drug activity for routine screening was read as the presence or absence of worms in the intestine on the 8th day of the infection. The number of worms in the untreated controls was counted. An actual count of the number of worms in the drug-treated groups was not made unless it was evident by subjective appraisal that there had been a reduction in number. The worms in mice may be counted quite simply and accurately by pressing the intact intestine between two glass plates and using low magnification.

For routine purposes the test compounds were mixed in the diet (Purina brand chicken mash) of the test animals (mice) and offered to them in hoppers designed to reduce wastage to a minimum. These hoppers are metal ointment cans of 4

ounce capacity with five holes one-half inch in diameter in the lid. A circle of one-quarter inch mesh hardware cloth is placed on top of the food in the hoppers to discourage the mice from attempting to dig into the food or scoop it out with their paws.

In a preliminary test a new compound is first tested at 0.5%, on a weight basis, in the diet of a group of three infected mice from the time of inoculation to the time of autopsy 8 days later. By treating during this entire period, activity of a drug against either the tissue or the intestinal stages may be detected. While it is true that the worms have just reached maturity by this time and have resided in the intestine only a few days, it was considered inadvisable to wait until later to make the autopsy for fear of confusing worm elimination due to acquired immunity with elimination due to drug action. If an entire group of mice is killed due to toxicity, the compound is retested at lower doses. The dosage is dropped in successive tests until a dose that is just tolerated is reached. A compound is reported as being inactive at maximum tolerated doses or at 0.5% drug-diet.

To prevent possible selection between particles of food and drug on the part of test animals, all drugs were finely ground in a mortar before being added to the diet. Food intakes were determined so that average daily drug intakes could be calculated.

All drugs are studied by special tests to determine the relative degree of activity, the stage of the infection susceptible to it, the best method of administration, and whether or not it is active against other species of worms and in other hosts.

Results:

Standard compounds:

For comparative purposes, many standard anthelmintics were tested, although not all of the more useful compounds (for example, chloroform, carbon tetrachloride, tetrachlorethylene, etc.) are suitable for drug-diet administration over a period of time because of their volatility. None of the compounds tested were active, although all were tested at approximately maximum tolerated doses. The drugs tested are as follows:

Drug	Dose in % Diet	No. Mice
Arecoline HBr	0.5	8
β-naphthol	0.5	9
Crystal violet	0.5	9
Gentian violet	0.3	6
Emetine	0.01	3
Ficin	0.5	3
Hexyl resorcinol	1.0	15
Phenothiazine	1.0	9
Santonin	0.4	3

That *N. muris* is not completely insensitive to standard anthelmintics is shown by the results of various trials using single oral doses of some drugs. The extensive trials of Whitlock and Bliss (1943) have shown that the ED_{50} (the dose which eliminates about 50% of the worms) of carbon tetrachloride against *N. muris* in rats is about 1 cc per kilogram of body weight (0.12–0.13 cc per rat). The same

dose of tetrachlorethylene eliminated about 80% of the worms from rats (Rogers, 1944). Four cc of a 1% solution of hydrogen peroxide administered to rats weighing about 150 g. eliminated about 60% of the worms harbored (Schwartz and Porter, 1937).

Our own limited experience with single oral dose administration of standard anthelminthics in *N. muris* infections is indicated in Table 2. There is little question but that carbon tetrachloride is active, however, the activity of the other compounds is not as clear-cut. For example, tetrachlorethylene seemed quite active in test H101 but less so in test H103B. Hexylresorcinol and phenothiazine also varied

TABLE 2.—*The action of standard anthelmintics when given as single oral doses to mice infected with* N. muris. *Treatment given on 7th day of infection*

Drug	Test No.	Drug dosage mg./kg.	Suspending medium	No. of mice	Av. worm count	Av. worm count as % of control count
Carbon tetrachloride	H101	2000	Peanut oil	4	10	12
		1000	"	4	27	32
		500	"	2	56	68
		0		3	84 (SE = 43.1) *	
	H103B	3000	"	9	20	12
		2000	"	10	45	27
		1000	"	10	105	62
		0		14	170 (SE = 29.1)	
Tetrachloroethylene	H101	3000	"	3	3	4
		1000	"	3	81	97
		0		3	84 (SE = 43.1)	
	H103B	4000	Cotton-seed oil	4	77	45
		3000	"	8	149	88
		0		14	170 (SE = 29.1)	
Hexylresorcinol	H23	1000	agar	toxic		
	H102	500	Peanut oil	5	14	33
		250	"	4	33	78
		0		7	42 (SE = 15.5)	
	H103B	800	"	7	160	95
		500	"	8	144	85
		250	"	3	163	97
		0		14	170 (SE = 29.1)	
Phenothiazine	H102	3000	"	4	5	12
		2000	"	5	2	5
		0		7	42 (SE = 15.5)	
	H103B	4000	Cotton-seed oil	6	109	64
		3000	"	7	121	71
		0		14	170 (SE = 29.1)	
β-Naphthol	H103A	500	Peanut oil	2 toxic	68	49
		250	"	4	112	80
		0		10	141 (SE = 20.1)	
Santonin	H103A	1000	"	1 toxic	52	37
		500	"	3	140	100
		0		10	141 (SE = 20.1)	

* S.E. = standard error of the mean.

considerably from one test to another. At best the action of β-naphthol and santonin was very slight. In addition, the following compounds (with maximum doses used given in parenthesis) were tested without effect: gentian violet (500 mg./kg.), thymol (1000 mg./kg.), aspidium (3000 mg./kg.), n-butyl chloride (1000 mg./kg.), hexachlorethane (500 mg./kg.) and arecoline hydrobromide (250 mg./kg.). It should be remembered that the infected mice were neither prepared in any special way for the treatment nor were they purged following treatment which might explain, at least in part, the failure of these drugs to show more effect.

While the failure of standard anthelminthics to exhibit much effect on *N. muris* was discouraging, it still seemed worth exploring the possibilities of using *N. muris*

TABLE 3.—*Effect of trichloroacetamide on infections with* N. muris *in mice when administered in the diet from the time of inoculation to the time of autopsy on 8th day after inoculation.*

% Drug in diet	No. trials	No. mice neg. / No. treated	Av. worm count Test goups / Control goups
0.5	9	52/52	0/110
0.2–0.25	17	49/57	1/110
0.1	18	17/47	9/110
0.05	2	0/9	+/+*
0.05	1	0/6	4/121
0.025	1	0/6	36/121

* + = many worms.

infections in drug screening by testing a number of compounds at random. At the time of writing this paper, something over 1500 compounds have been tested. The most interesting discovery was the activity of trichloroacetamide and related compounds. The effect of trichloroacetamide under different conditions is shown in Tables 3, 4, 5, 6. When the drug was administered at a concentration of 0.2% or more in the diet of 109 mice in 26 separate trials, all but 8 of the mice were without worms on the 8th day after inoculation (Table 3). With 0.1% of drug in the diet, some of the 47 mice treated were without worms on the 8th day and the average number of worms was only about 10% of the average number found in the untreated controls. Lower doses were only slightly effective.

It is evident (Table 4) from trials with treatment restricted in different periods of the infection that trichloroacetamide is most effective against developing worms and almost or completely ineffective against the mature worms. However, treatment for only a single day whether it was the 1st, 2nd or 3rd day had no effect. Two or three days' treatment was partially effective, if started on the 1st day of the infection but not if started later. Longer treatment, if started as early as the 2nd or 3rd day of the infection had some effect. It is evident then that trichloro-

TABLE 4.—*Treatment with trichloroacetamide during different periods of infections with* N. muris *in mice*

Test No.	Days treated (inclusive)	Drug dosage in % diet	No. of mice surviving on 8th day	No. of neg. mice	Av. worm count 8th day*
H56	1–4	1.0	6/6	6	0
		0.5	5/6	3	3
		0.2	6/6	2	4
		0.1	6/6	1	16
		0.05	6/6	1	7
	3–4	0.5	6/6	6	0
	5–8	1.0	3/4	0	31
	0	0	5/5	0	121
H57	4–8	0.5	6/6	0	+
	3–8	0.5	6/6	0	8**
	2–8	0.5	4/6	2	1**
	1st	0.5	6/6	0	+
	1–2	0.5	6/6	0	18
	1–3	0.5	6/6	2	15
	1–4	0.5	5/6	1	ca. 50
	2nd	0.5	4/6	0	+
	2–3	0.5	6/6	0	+***
	2–4	0.5	4/6	0	+***
	3rd	0.5	5/6	0	+
	3–4	0.5	4/6	0	+
	4th	0.5	6/6	0	+

* A + means worms not actually counted but judged to be about as numerous as in the controls.
** Worms may have been dead.
*** Immature or stunted worms.

TABLE 5.—*Action of trichloroacetamide when administered as a single oral dose each day to mice infected with* N. muris

Test No.	Drug dosage mg./kg.	Days treated (inclusive)	No. of mice surviving on 8th day	No. mice without worms on 8th day	Av. worm count 8th day*
H59	800	1–7	5/6	5	0
	400	1–7	5/6	5	0
	200	1–7	6/6	3	1
	100	1–7	5/6	1	ca. 50
	50	1–7	4/6	0	+
	0	0	8	0	+
H65	1000	1st	5/6	4	2
	1000	6th	6/6	2	ca. 25
	0	0	6	0	144
H71B	1000	1st	6/6	0	18
	800	1st	6/6	0	+
	400	1st	6/6	0	ca. 60
	200	1st	5/6	0	+
	100	1st	6/6	0	+
	1000	6th	6/6	0	+
	0	0	5	0	110

* + = count judged to be equal to controls. If + recorded for control this means many worms (i.e. 100–200).

acetamide will prevent infections with *N. muris* in mice but will not eradicate established infections.

If trichloroacetamide is administered as a single dose each day, rather than continuously as an ingredient of the diet, as little as 200 mg./kg. per day has a marked effect (Table 5). Since the daily drug intake on a 0.2% diet is about 400 mg./kg., it is possible that the drug is somewhat more active when administered as a single dose each day, although it would obviously take extensive trials to prove that this difference is significant.

Trichloroacetamide seems to have roughly the same magnitude of activity against *N. muris* in rats as in mice (Table 6).

The action of compounds related to trichloroacetamide is shown in Table 7. Several of these were about as active as trichloroacetamide but none more so.

About 125 other compounds exhibited slight or inconsistent activity. These compounds are undergoing further investigations. The group includes Hetrazan (Hewitt, et al, 1947) a fact which suggests that other members of this group may exhibit important activity in infections other than those with *N. muris*.

Discussion

The *in vivo* method used for screening compounds for anthelmintic activity is easy and permits the examination of relatively large numbers of new compounds which may be available only in small quantities.

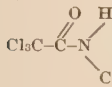
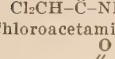
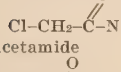
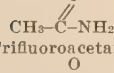
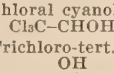
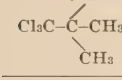
Most investigators would probably agree that any chemical compound which

TABLE 6.—*Action of trichloroacetamide on infections with* N. muris *in rats. Drug-diet administration from time of inoculation to time of autopsy at 8 days*

Drug dosage in % diet	No. of rats with		
	No worms	Few worms	Many worms
0.5	5	0	0
0.2	0	4	2
0.1	0	3	3
0.05	0	2	4

eliminated or prevented the development of infections with *N. muris* is certainly interesting and deserves further investigation. But the question of whether results obtained with infections with *N. muris* will apply to other worm infections, particularly the important infections of domesticated animals, can only be answered by such investigations of a number of compounds active against *N. muris.*

TABLE 7.—*The effect on infections with* N. muris *of compounds related to trichloroacetamide. Treatment by drug-diet method from day of inoculation to time of autopsy 8 days later*

Compound	Test No.	Dosage in % diet	No. of mice	Av. worm* counts test/control
Trichloroaceto-p-toluide	H60	0.5	3	0/many
	H66	0.2	3	0/many
		0.1	3	0/many
	H71	0.1	2	4/109
		0.05	3	20/109
		0.025	2	31/109
	H96	0.5	3	1/112
		0.3	3	10/112
		0.1	3	few/112
		0.05	3	many/112
N-(Chloroacetyl)trichloroacetamide	H58	0.5	3	0/many
				
Chloral hydrate	H66	0.5	3	0/many
		0.1	3	7/many
$Cl_3C-CH(OH)_2$	H97	0.5	3	1/41
		0.3	3	20/41
		0.1	3	30/41
Chloral (stabilized with hydroquinone)	H84	0.5	2	0/70
CCl_3-CHO	H95	0.3	3	10/99
		0.1	3	4/99
Ethyl trichloroacetate	H95	0.3	3	0/99
$Cl_3C-COOC_2H_5$		0.1	3	5/99
Chloral alcoholate	H84	0.5	3	0/70
$Cl_3C-CH(OH)OC_2H_5$	H95	0.3	2	0/99
		0.1	1	many/99
	H97	0.1	3	21/41
Dichloroacetamide	H48	0.5	3	many/198
				
Chloroacetamide	H50	0.5	toxic	
	H54	0.1	2	many/many
				
Acetamide	H83	0.5	2	many/52
				
Trifluoroacetamide	H73	0.4	2	many/many
		0.1	2	many/many
		0.025	1	many/many
				
Chloral cyanohydrin	H84	0.1	1	many/70
$Cl_3C-CHOH-C\equiv N$				
Trichloro-tert.-butyl alcohol	H88	0.1	3	many/many
				

* "Many" worms = between 70 and 200—"few" worms = less than 30.

Trichloeoacetamide has been tested in these Laboratories against six other helminths without effect (Table 8). This failure of the activity to carry over is disappointing but as a single example is not discouraging, since one might expect the greater number of compounds to be limited rather than broad in their range of activity.

No other compounds gave as spectacular results in *N. muris* infections as did trichloroacetamide and certain related compounds. The only evidence to date that any of the compounds that gave doubtful or inconsistent results are of interest is that Hetrazan has been shown to have promise in other infections. Only further investigations will clarify the problem in respect to the hundred-odd compounds giving results resembling those seen for Hetrazan. It is reassuring to know that a drug like Hetrazan would not have been completely overlooked. On the contrary, failure of a number of standard anthelmintics to have any effect on *N. muris* infections might suggest to many that the test described here will overlook compounds of interest. However, it has become generally agreed by investigators in chemotherapy that it is impossible to design a screening procedure that will not overlook certain types of drug activity, because of either the techniques used or worm species employed. In fact, the "critical" test of Hall (1921) using infec-

TABLE 8.—*Action of trichloroacetamide against miscellaneous helminth infections*

Infection	Host	Treatment	Results
Strongyloides ratti 1000 larvae subcutaneously	Rats: 150–180 g. 3 per group	Drug-diet from day of inoculation to time of autopsy on 8th day	
		Trichloroacetamide 0.5%	Inactive
		Hexylresorcinol 0.5%	"
		β-Naphthol 0.5%	"
		Phenothiazine 1.0%	"
Litomosoides carinii natural infections	5 cotton rats	0.5% Drug in diet for 10 days	No effect on either microfilaria or adults
Oxyurids natural infections	12 mice	0.5% Drug in diet for 3 days	Inactive
Schistosoma mansoni 150 cercariae intraperitoneally	9 mice	0.5% Drug in diet starting 1 day after inoculation and continued for 15 days	Development of worms not affected
	10 mice	0.5% Drug in diet from 40th to 47th day of infection	Adult worms not affected
Hymenolepis nana natural infections	12 mice selected at random	0.5% Diet for 3 days	10/12 mice with many large worms: therefore, drug inactive
Trichinella spiralis experimental infections	18 mice	0.5%–1.0% Drug diet during intestinal phase	No effect on adult worms
	18 mice	0.5%–1.0% Drug diet during larval migration or while larvae in muscles.	No effect on migrating or encysted larvae.

tions in dogs failed to reveal the important properties of phenothiazine (Swales, 1940) simply because these infections are not susceptible to this drug. Phenothiazine is also inactive *in vitro* against *Ascaris lumbricoides* (Baldwin, 1943). The test described here is designed to find a compound which can be administered orally by means of the drug-diet method with dosage continuing for a considerable period of time, while tests described in the literature were most characteristically designed to detect activity in compounds given as large, single, oral doses by a stomach tube or similar instrument.

The small number of compounds showing consistently good activity and the larger number giving questionable results is somewhat puzzling but is not out of line with the results of other screening programs. Schubert (1948) in his work with *Schistosoma mansoni* infections in mice found out of over 400 compounds tested, only 3 (all antimonials) which gave good results. Seven compounds, only 3 of which were not antimonials, reduced the worm burden somewhat and 33 compounds were of "slight or doubtful permanent value." Excluding the antimonials, which, of course, were known to be active against *S. mansoni* before Schubert's

work, only 21 out of over 400 compounds, or about 5%, were found to have any effect at all and in most cases this effect was slight or doubtful. This is essentially the same as our results where about 125 out of 1500, or 8% of the compounds, have given questionable results.

Just how well infections with *N. muris* in mice satisfy the requirements for the bio-assay of anthelmintics depends on the interpretations one wishes to make Whitlock (1943) has analyzed the populations of *N. muris* found in rats inoculated with about 500 larvae. The mean number of worms in 11 groups of rats varied from 117.0 to 371.2. This was perhaps due to a lack of uniformity in the lots of rats or in the batches of larvae used for inoculation. However, within groups the number of worms established was quite uniform; the coefficients of variation ranging from 11.3–26.4. Whitlock concluded that this uniformity of infection within samples permits the accurate determination of the percent of worms eliminated by an active drug from parallel worm counts in treated and untreated infected rats. The greater variation in the number of worms established in mice (Table 1) prohibits as accurate a determination of this sort to be made. Therefore, it would seem that, while the infections in mice are satisfactory for routine screening, because of the advantages already discussed, and while they can be used with reasonable satisfaction for assay purposes, as illustrated by the results of trials with trichloroacetamide, it would be best to use infections in rats where more accurate assays are desired.

The test might perhaps have been made more sensitive by using a reduction in egg output rather than worm burden as the endpoint. However, what might have been gained in increased sensitivity would have been lost in the reduced number of compounds that could be investigated in a given time since the making of quantitative egg counts is more time-consuming than the autopsy and worm counting methods described here.

The principal criticism that might be leveled at the use of mice as the host in these studies is that, since the species is not the most favorable host, the worms would be eliminated more easily by drug treatment, thus giving many false positives. It is possible that some of the inconsistent results obtained with certain compounds were related to this factor but in general the authors have been tremendously impressed with the tenacity of this species of worm in mice in spite of the presumably adverse conditions that must have resulted in many cases by the drug treatment.

The discovery of the hitherto unknown properties of trichloroacetamide and related compounds lends encouragement to continuing the search for better anthelmintics using the techniques described here, since other equally active compounds may be discovered and these may be sufficiently broad in action to be practical drugs in important infections.

SUMMARY AND CONCLUSIONS

1. An *in vivo* screening test for anthelmintics has been described which uses infections with *N. muris* in mice. The drug-diet method of administration was used with treatment extending continuously from the time of inoculation to the time of autopsy 8 days later.

2. This test requires only 0.3 g. of compound for a preliminary trial.

3. Over 1500 compounds have been tested with only one group, namely, trichloroacetamide and some related compounds, showing spectacular effects. About 125 other miscellaneous compounds had doubtful or inconsistent effects on the infections.

4. Trichloroacetamide is not active against *Strongloides ratti* or *Trichinella spiralis* in rats; *Litomosoides carinii* in cotton rats; *Hymenolepis nana* or oxyurids in mice; nor *Schistosoma mansoni* in mice.

References

Africa, C. M. 1931a Studies on the activity of the infective larvae of the rat strongylid, *Nippostrongylus muris*. J. Parasit. **17**: 196–206.

———1931b Studies on the host relations of *Nippostrongylus muris*, with especial reference to age resistance and acquired immunity. J. Parasit. **18**: 1–13.

Baldwin, E. 1943 An *in vitro* method for chemotherapeutic investigation of anthelmintic potency. Parasitol. **35**: 89–111.

Chandler, A. C. 1937 Studies on the nature of immunity to intestinal helminths. V Experiments on the role of the skin in parenteral immunity, and further experiments on passive immunization in *Nippostrongylus* infections in rats. Am. J. Hyg. **26**: 292–308.

Findlay, G. M. 1939 Recent Advances in Chemotherapy. 523 pp. London.

Graham, G. L. 1934 Resistance studies with the nematode, *Nippostrongylus muris*, in laboratory rats. Am. J. Hyg. **20**: 352–372.

Hall, M. C. 1921 Carbon tetrachloride in the removal of parasitic worms, especially hookworms. J. Agr. Res. **21**: 157–175.

Hall, M. C. and Shillinger, J. E. 1925 Tetrachlorethylene, a new anthelmintic. Am. J. Trop. Med. **5**: 229–237.

Hardwood, P. D., Jerstad, A. C. and Swanson, L. E. 1938 J. Parasit. **24** (Suppl.): 16.

Hewitt, R. I. Wallace, W. S., White, E. and Subbarrow, Y. 1947 Experimental chemotherapy of filariasis. I Experimental methods for testing drugs against naturally acquired filarial infections in cotton rats and dogs. J. Lab. and Clin. Med. **32**: 1293–1303.

Lamson, P. D. and Brown, H. W. 1936 Methods of testing the anthelmintic properties of ascaricides. Am. J. Hyg. **23**: 85–103.

Leonard, V. 1924 Hexylresorcinol. J. Urol. **12**: 585.

Porter, D. A. 1935 A comparative study of *Nippostrongylus muris* in rat and mice. Am. J. Hyg. **22**: 444–466.

Rogers, W. P. 1944 Studies on the anthelmintic activity of hexylresorcinol and tetrachlorethylene. Parasitol. **36**: 98–109.

Schwartz, B. and Alicata, J. E. 1934 The development of the trichostrongyl, *Nippostrongylus muris*, in rats following ingestion of larvae. J. Wash. Acad. Sci. **24**: 334–338.

Schwartz, B. & Porter, D. A. 1937 Tests with hydrogen peroxide as an anthelmintic. Rev. med. Trop. y Parasit. **3**: 11–24.

Schubert, M. 1948 Conditions for drug testing in experimental *Schistosomiasis mansoni* in mice. Am. J. Trop. Med. **28**: 121–136.

Swales, W. E. 1940 The use of phenothiazine in veterinary parasitology. Canadian J. Compar. Med. **4**: 333–341.

Whitlock, J. H. 1943 Characteristics of the population available for bio-assay of anthelmintics in *Nippostrongylus muris* infection in albino rats. J Parasit. **29**: 42–47.

Whitlock, J. H. and Bliss, C. I. 1943 A bio-assay technique for anthelmintics. J. Parasit. **29**: 48–58.

Yokagawa, S. 1920 A new nematode from the rat. J. Parasit. **7**: 29–33.

Yokagawa, S. 1922 The development of *Heligmosomum muris* Yokagawa, a nematode from the intestine of the wild rat. Parasitol. **14**: 127–166.

INTESTINAL PHASE OF *TRICHINELLA SPIRALIS* (OWEN, 1835) RAILLIET, 1895

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INTRODUCTION

During the routine of experimental work on *Trichinella spiralis* certain trends of distribution of these parasites in the small intestine of rats were repeatedly observed. Modern textbooks on parasitology discuss the intestinal phase only superficially, and investigators in this particular field seem to have divergent opinions on such problems as distribution, elimination of males, and sex ratio. Hoping that more light might be cast upon this part of the life cycle, the author felt justified in making an investigation of the distribution of maturing and adult male and female trichinae in the digestive system of rat hosts after experimental infection.

MATERIALS AND METHODS

Laboratory-bred rats were maintained on a diet of milk, dog-biscuit and water. Feeding, except for water, was omitted on the morning before experimental infection and again on the morning before autopsy of infected animals. The influence of age factors was avoided throughout the work by utilizing only 4 to 6 months old rats.

The trichina larvae were obtained from infected stock rats as described in the preliminary experiment (Gursch, 1948). Doses of 1280 larvae were prepared by direct count and concentrated by centrifugation. By means of a stomach tube, the larvae were administered to anesthetized rats in such sequence that there was ample time to complete individual experiments. At the periods indicated (Fig. 1), each rat was killed and the stomach and intestine were removed. The small intestine was then divided into 4 equal parts, a division which seemed to simplify the subsequent presentation of linear distribution of the worms. Each of these quarter sections, as well as the entire stomach, large intestine and cecum, was placed in a separate dish with warm 0.85% saline (approx. 37° C.), and subsequently cut open and incubated for 1 hour at 37° C. Then the mucosa was stripped from the muscularis and well broken up by vigorous shaking. From each separate section of the digestive system, the worms were counted directly and the total number for the entire gut computed.

This experiment was repeated with doses of 5000 larvae, which were estimated by dilution counts. Here 10 one cubic cm samples were drawn from a suspension of the larvae in water. The counts of the individual samples were averaged, and the number of cubic cm of the solution calculated for a 5000 larvae dose. The numbers of worms recovered after certain periods of infection were estimated by dilution counts.

Another method used to determine the distribution of *T. spiralis* was that of

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serial sectioning four 1 cm long pieces from the small intestine. From each quarter section, 1 cm was cut from the posterior end, fixed, and later sectioned for examination of the worms in situ. This revealed the distribution not only in respect to the length of the gut, but also to the depth of penetration into its wall.

RESULTS AND DISCUSSION

1. *Distribution of* Trichinella spiralis *in the Digestive System of Rats*

The average percentage of the total numbers of parasites recovered from rats infected with doses of 1280 larvae has been plotted against periods of infection (Fig. 1). The graph shows a loss of 47% during the first 24 hours after experimental infection. Even after so short a period as 2 hours, living and dead parasites were found in the large intestine, and 13% of the worms were lost.

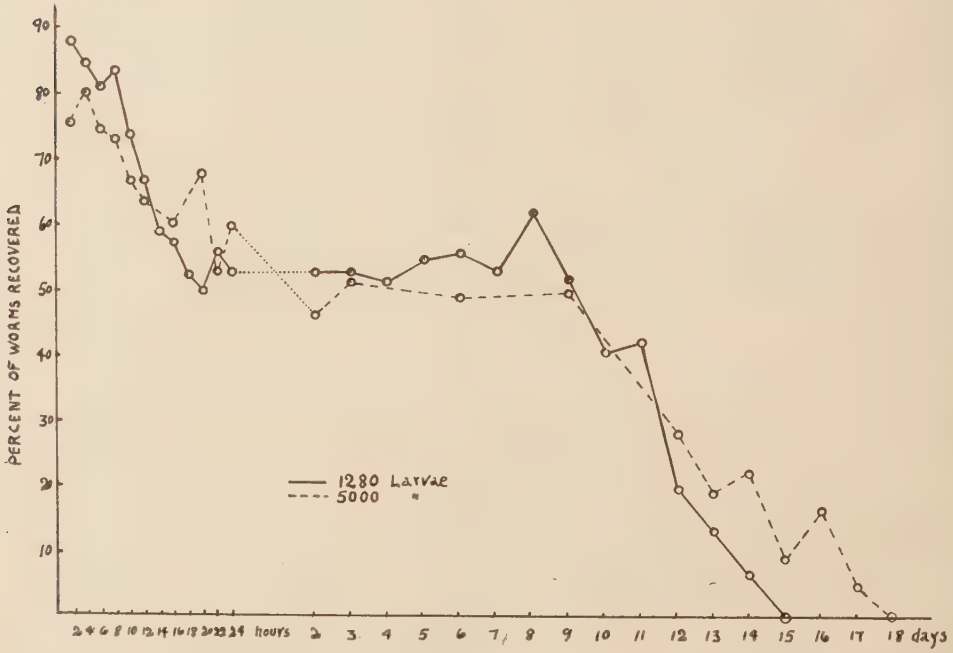


FIG. 1. Recovery of *Trichinella spiralis* from the digestive system of rats infected with 1280 and 5000 larvae respectively. 3 rats were used for each period of infection comprising a total of 87 rats. (All data are available in tabulated form at the Parasitology Department of the State University of Iowa.)

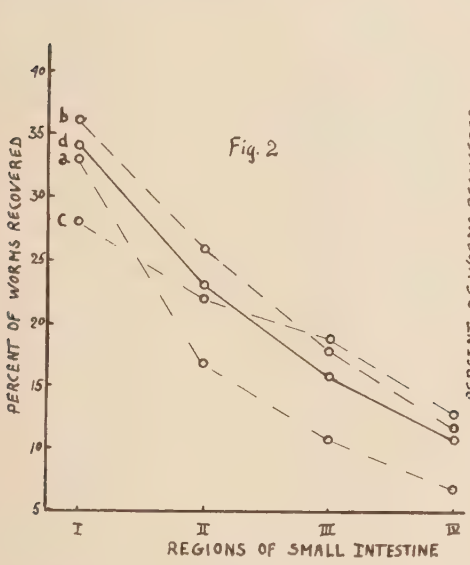
Between the first and approximately the 9th day of infection, the data show an apparent stabilization of the number of adults in the intestine (Fig. 1). This is the period in their life cycle which includes the most vigorous activities of these parasites, namely, growth, sexual maturity, copulation, development of young and deposition of progency.

After the 9th day, there was a rapid loss of worms until complete disappearance of adults by the 15th day of infection. This constituted the third and last period of the intestinal infection.

The results obtained from 5000 larvae infections were also plotted in Fig. 1, and formed a curve similar to that obtained with the smaller doses. Here approxi-

mately 24% of the worms were lost after 2 hours of infection. After the 9th day there was a gradual decline in the number recovered. None was found on the 18th day.

The distribution of worms in the small intestine of rats infected with 1280 and 5000 larvae showed definitely that a greater number was concentrated in the first quarter of the small intestine. Subsequently the number decreased with each successive section. This linear distribution has been plotted according to the periods indicated (curves a, b, c, d in Fig. 2). It seems that irrespective of the length of infection, the distribution of worms in the small intestine is essentially the same. Roth (1938a) concluded that in the intestine of guinea pigs the adult worms prefer the posterior region.



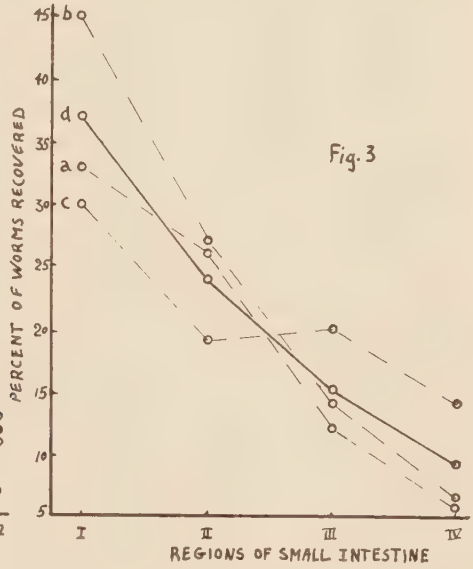


FIG. 2. *T. spiralis* recovered from each quarter section of small intestine of rats infected with 1280 larvae. (Worms recovered from stomach, cecum and large intestine not included). Average number from the following periods:

a. 2 hrs to 14 hrs.
b. 16 hrs to 9 days.
c. 10 days to 14 days.
d. 2 hrs to 14 days.

Fig. 3. *T. spiralis* recovered from each quarter section of small intestine of rats infected with 5000 larvae. (Worms recovered from stomach, cecum and large intestine not included). Average number from the following periods:

a. 2 hrs to 14 hrs.
b. 16 hrs to 9 days.
c. 10 days to 17 days.
d. 2 hrs to 17 days.

Throughout the infection, worms were found in the stomach, the cecum, and the large intestine. It was surprising to find occasionally, in rats infected with 5000 larvae, a few vigorously moving worms in the stomach as long as 15 to 16 days after infection. All appeared to be in the same stage of development as those in the small intestine. No explanation can be offered except that these sexually mature

worms either had never reached the intestine or had returned from the intestine to the stomach.

Only a few worms were recovered from the large intestine and cecum after the 2 and 4 hour period. However, greater numbers of worms were found in subsequent periods of 6 to 20 hours, and it seems probable that these larvae were on their way out. (Tabulated data are available at the Parasitology Department, State University of Iowa).

The data obtained by serial sections of 1 cm portions of the small intestine have been tabulated in Table 1. The numbers of worms were calculated for each quarter section by multiplying the number of worms found in 1 cm long piece of intestine by the length of the particular section from which it was cut. When the average percentages of worms recovered from the small intestine of rats in-

TABLE 1.—*Distribution of* Trichinella spiralis *obtained by serial sectioning of one centimeter from each quarter of small intestine after one hour to 17 days of infection. Each rat was given 5000 larvae.*

No. of rats	Wt. in gms	Time of inf.	Length of ¼ sect. in cm	Number of worms in each quarter section of small intestine				Percent recovered
				I	II	III	IV	
		Hours						
1	145	1	15.0	300	135	150	0	11.7
1	164	2	15.0	405	1530	705	660	66.0
2	155	4	15.0	1641	525	373	146	53.7
2	150	6	15.0	645	1170	990	300	62.1
2	153	8	15.0	2047	1115	353	375	77.8
2	167	10	18.0	894	888	210	68	41.2
2	168	12	17.0	1326	532	300	264	48.4
2	158	16	16.0	1552	569	182	51	47.1
2	169	20	16.8	1286	630	365	147	48.6
2	146	22	15.5	1004	575	239	205	40.5
2	150	24	15.5	1167	513	351	94	42.5
1	215	48	20.0	380	280	440	140	24.8
2	180	72	19.0	535	298	337	94	25.3
2	180	144	19.3	336	491	257	111	23.9
2	210	216	20.8	841	444	250	140	33.5
		Days						
2	193	12	18.5	145	48	352	95	12.8
1	220	13	22.0	44	...	...	...	0.9
2	190	14	18.0	612	279	135	81	22.1
1	200	15	20.0	100	...	...	...	2.0
2	215	16	21.0	300	219	144	67	14.6
1	240	17	24.0	...	...	...	...	...

fected with 5000 larvae were calculated by both the dilution and sectioning method, the numbers and distribution obtained by the two techniques were quite similar.

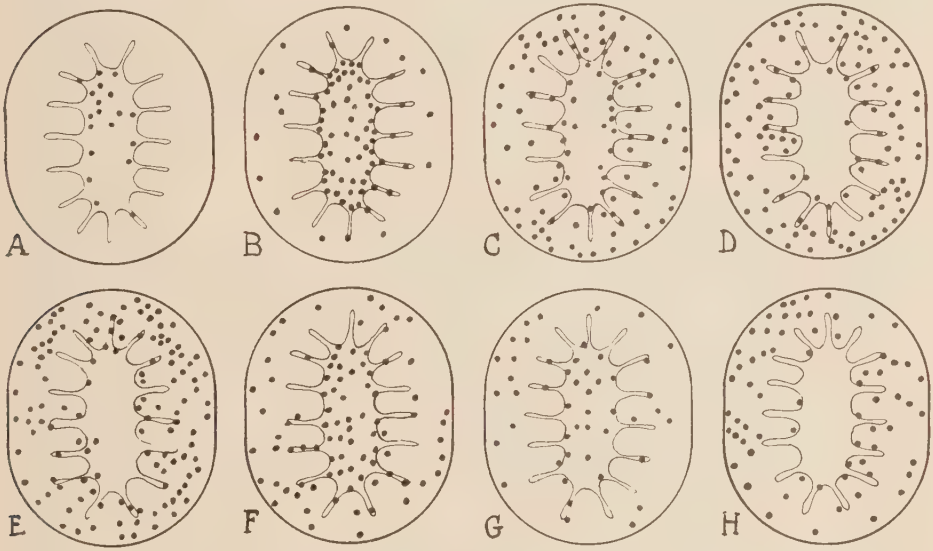
2. Distribution of Trichinella spiralis *in the Mucosa of Small Intestines of Rats*

Microscopic examination of serial sections of portions of infected small intestine revealed the location of worms in the wall as well as in the lumen. The location of worms in each cm section was recorded on a single diagrammatical cross-section of a gut. A few of such composite cross-sections (1, 2, 4, 8, 16, 22, 24 hour and 14 day infections from first quarter sections) have been presented in Plate I. The object of these selections was to show the exact location of the worms at various periods throughout the course of infection.

In as short a period as one hour (Plate I, Fig. A), many worms had already left the stomach and some had already reached the third quarter of the small intestine (Table 1). At 2 hours many had penetrated the mucosa, and a few had even reached the muscularis (Plate I, Fig. B). After 4 hours of infection only a comparatively few remained in the lumen (Plate I, Fig. C).

After 20 hours of infection most worms were again located in the lumen of the intestine. The sections taken from the intestine of the rat after 22 and 24 hour infections (Plate I, Figs. E,G) include many cross-sections of worms in the lumen which were more or less clustered together at different locations. It is possible that this emergence into the lumen is for copulation. However, as recently as 1933 Doerr and Menzi suggested that copulation must take place in the mucosa because they never observed mature females and males in the lumen.

After 2 days, the worms had repenetrated the mucosa with few adults in the lumen. Some were lodged in the crypts, but most of the females and males penetrated deeply. The fact that males were also found close to the muscularis does not agree with the opinion of Kreis (1937), who believed that only sexually matured females bore into the intestinal villi.



PLATE

PLATE I

Composite diagrams of serial sections of one centimeter of small intestine taken at the posterior end of the first quarter section. Dots indicate approximate position of each worm.

FIG. A. 1 hour infection
FIG. B. 2 hour infection
FIG. C. 4 hour infection
FIG. D. 8 hour infection
FIG. E. 16 hour infection
FIG. F. 22 hour infection
FIG. G. 24 hour infection
FIG. H. 14 day infection

3. *Destruction of Intestinal Mucosa of Rats by* Trichinella spiralis

T. spiralis is a tissue parasite because the larval and the adult phases are spent for the most part in muscle tissue and in the glandular tissue of the small intestine, respectively. According to Kreis (1937), the worms live in the intestinal mucosa without destroying the cells. Yet Heller (1933) designed experiments in which he proved that trichinae must derive their food from the intestinal mucosa

of the host. This is indirectly substantiated by McCoy (1936), who concluded that the worms feed upon living host tissue.

During an investigation of the distribution of trichinae in the digestive system of experimentally infected rats, serial sections were obtained showing nearly entire worms in the mucosa (Plate II). In as short a period as 2 hours, a few larvae were already securely lodged between the villi of the small intestine and were be-

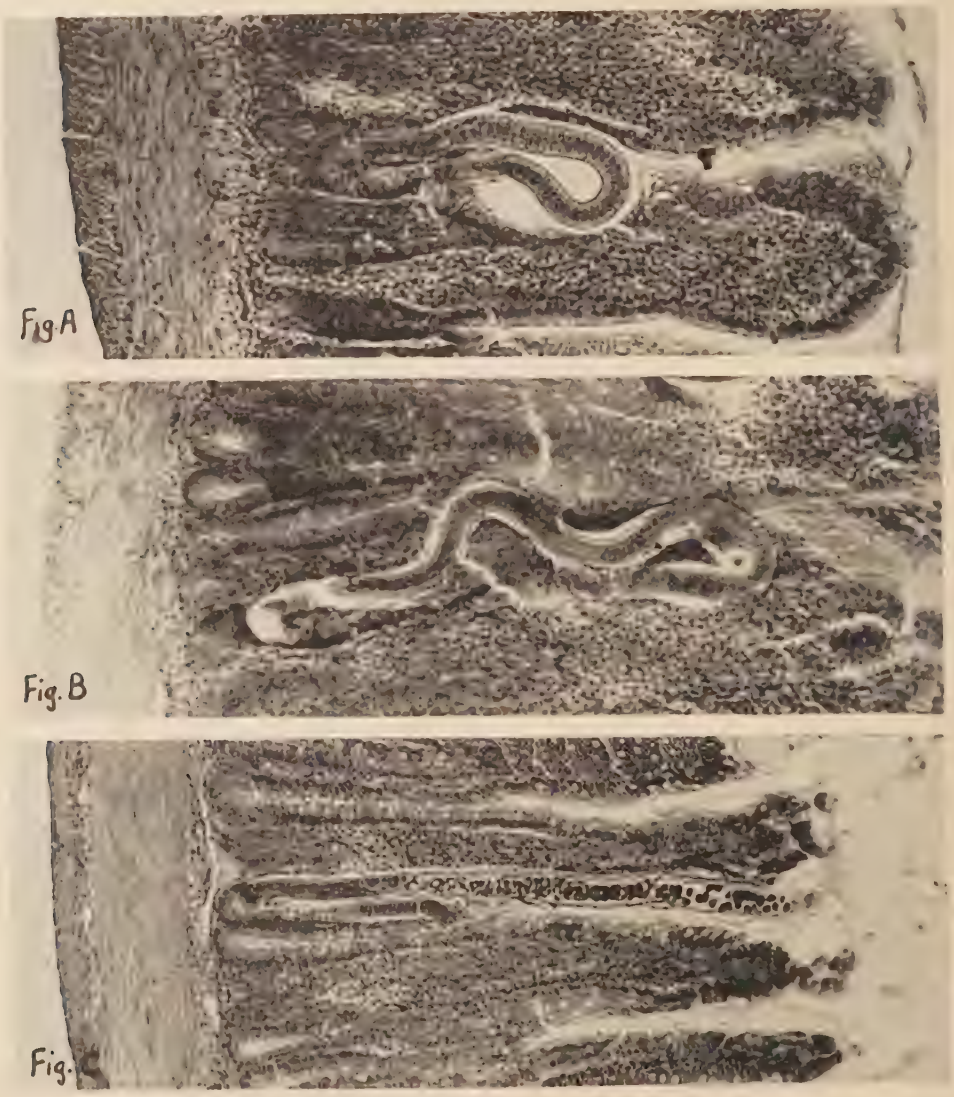


PLATE II

FIG. A. Larva of *T. spiralis* in mucosa of small intestine of rat killed 8 hours after experimental infection.

FIG. B. Larva of *T. spiralis* in mucosa of small intestine of rat killed 8 hours after experimental infection.

FIG. C. Adult female of *T. spiralis* in mucosa of small intestine of rat killed 16 days after experimental infection.

ginning penetration of glandular tissue (Fig. A). The subsequent growth of the maturing worms caused extensive destruction of the villi (Figs. B,C). Many adults reached the muscularis and upon contact turned about, which caused still more tissue destruction of the intestine.

4. *Sex Ratio of* Trichinella spiralis *in the Intestine of Rats*

Christenson (1927) and McCoy (1931) believed that the sex ratio is 1 to 1. Roth (1938b), however, determined both sexes microscopically according to Bugge's (1934) characterization before feeding the trichina larvae to guinea pigs, and found a sex ratio of approximately 2 females to 1 male. However, such sex identification at this larval stage of development was not practical for extensive infection experiments.

In this work, therefore, sex determinations were made of trichinellas recovered from 47 of the rats infected with 1280 larvae over a period of time ranging from 2 to 14 days (Table 2). The calculated ratios revealed that through the main period of reproduction, the 2nd to 9th day inclusively, the females outnumbered the

TABLE 2.—*Average numbers of female and male* T. spiralis *recovered from digestive system of rats infected with 1280 larvae*

Days of inf.	Small intestine I		II		III		IV		Cecum L. int.		Total average		Sex ratio
	F	M	F	M	F	M	F	M	F	M	F	M	F/M
2	290	126	62	32	52	29	32	8	19	11	456	206	2.2
3	263	124	82	35	34	25	52	25	2	3	433	212	2.0
4	147	66	131	55	87	37	45	31	17	5	427	194	2.2
5	208	84	131	77	63	20	62	27	21	8	485	217	2.2
6	189	86	132	69	131	59	58	27	26	12	536	253	2.1
7	151	93	97	43	106	49	66	30	28	5	448	220	2.0
8	182	77	121	72	173	78	64	35	11	4	551	266	2.1
9	190	91	105	59	49	30	33	19	14	10	391	209	1.9
10	102	51	80	51	53	64	30	24	58	18	323	208	1.6
11	73	47	40	43	58	50	53	35	89	54	313	229	1.4
12	71	23	25	30	27	25	39	29	52	34	214	141	1.5
13	43	21	58	30	63	34	11	6	10	15	185	106	1.7
14	36	24	13	22	4	7	5	4	6	5	64	62	1.0

males approximately 2 to 1. After the 9th day, the number of females decreased more rapidly than that of males so that the sex ratio was nearly 1 to 1 on the 14th day of infection. It may be reasonably assumed that the larvae lost during the first few hours of infection were females and males in a similar proportion of approximately 2 to 1.

A knowledge of the sex ratio should be considered in the computation of reproductivity of females. Investigators have computed such potentiality only on the basis of larvae-larvae ratios, that is the ratio of the total number of larvae which develop in muscles to the number originally given to the host. McCoy (1932a) computed the reproduction of larvae by female trichinellas in rat hosts as ranging from 200 to 400 on the assumption that half the larvae fed were females. This is substantiated by Wolffhügel (1938), who infected a rat with 2 larvae and obtained 200 larvae from 1 female. Such figures are rather low when compared with the ones obtained by Nolf (1937), who implanted single gravid females in the intestine of rats and found that as many as 1112 larvae were produced by 1 female. McCoy (1932b) obtained an average ratio of 1500 larvae in monkeys, in the intestine of which the adult trichinae live considerably longer than in rats. Roth (1938b) obtained 1000 to about 2500 larvae per female from infections of

guinea pigs, a host in which the adult worms live approximately 4 to 5 weeks in the small intestine.

SUMMARY AND CONCLUSION

The results of these experiments demonstrated that in general the greatest percentage of recovery of *Trichinella spiralis* was from the first quarter section of the small intestine, and that the percentage of worms recovered from the following sections declined steadily.

The larvae penetrated the mucosa immediately after ingestion. After 22 and 24 hours of infection, the worms were found again in the lumen. Subsequently, females as well as males penetrated the mucosa and caused extensive destruction of villi.

After two days of infection, the adults recovered revealed a ratio of two females to one male. Even among those which were eliminated, that is, found in the cecum and large intestine, there were always more females than males.

REFERENCES

BUGGE, G. 1934 Trichinen im Darm. Arch. f. Tierheilkd. **68:** 24–32.

CHRISTENSON, R. O. 1927 The sex ratio of adult trichinae. *Science* **66:** 259.

DOERR, R. UND E. MENZI. 1933 Studien über den Mechanismus der Trichinelleninfektion. VIII. Mitteilung. Vergleichende Untersuchungen über die Empfänglichkeit der Ratte und des Meerschweinchens für die Infektion per os. Zentr. Bakt., Orig., I. Abt. **128:** 177–188.

GURSCH, O. F. 1948 Effect of digestion and refrigeration on the ability of *Trichinella spiralis* to infect rats. J. Parasit. **34:** 394–395.

HELLER, M. 1933 Entwickelt sich die *Trichinella spiralis* in der Darmlichtung ihres Wirtes? Zeit. Parasit. **5:** 370–392.

KREIS, H. A. 1937 Die Entwicklung der Trichinellen zum reifen Geschlechtstier im Darme des Wirtes. Zentr. Bakt.,Orig., I. Abt. **138:** 290–302.

MCCOY, O. R. 1931 The course of experimental trichiniasis infections in rats. J. Parasit. **18:** 123.

MCCOY, O. R. 1932a Size of infection as an influence on the persistence of adult trichinae in in rats. Science **75:** 364–365.

MCCOY, O. R. 1932b Experimental trichiniasis infections in monkeys. Proc. Soc. Exp. Biol. and Med. **30:** 85–86.

MCCOY, O. R. 1936 The development of trichinae in abnormal environments. J. Parasit. **22:** 54–59.

NOLF, L. O. 1937 The transplantation of gravid *Trichinella spiralis*. J. Parasit. **23:** 574.

ROTH, H. 1938a On the localization of adult trichinae in the intestine. J. Parasit. **24:** 225–231.

ROTH, H. 1938b Experimental studies on the course of trichina infection in guinea pigs. I. The minimum dose of trichina larvae required to produce infestation of the muscles; with an account of the potential productiveness of the female trichina. Amer. Jour. Hyg. **28:** 85–103.

ROTH, H. 1939 Experimental studies on the course of trichina infection in guinea pigs. II. Natural susceptibility of the guinea pig to experimental trichina infection. Amer. Jour. Hyg. **29** (Sect. D) : 89–104.

WOLFFHÜGEL, K. 1938 Wieviel eingekapselte Trichinen erzeugt ein Muttertier? Zeit. Fleisch-u. Milch-hyg. **48:** 301–302.

MILK AS A SOURCE OF SOME PROTECTION AGAINST THE ACQUISITION OF *TRICHINELLA SPIRALIS* IN MICE[1]

BERNARD B. RIEDEL[2]

Host resistance toward nematoid parasites is sometimes greatly affected by the presence of milk in the diet. Among the investigators who presented results to that effect are Ackert and Beach (1933) and Ackert and Riedel (1946). They found that the presence of skim milk in a diet increased the resistance of chickens to *Ascaridia galli*. Porter (1935) stated that a milk diet increased the susceptibility of rats to *Nippostrongylus muris*.

The purging value of milk in eliminating parasites has also been reported by several investigators. Spindler, Zimmerman and Hill (1944) found that heavily infected pigs kept on a milk diet alone for three to five days eliminated during this time from 61.36 to 100 percent of their whipworms, 90.75 to 100 percent of their nodular worms and from 0.0 to 94.11 percent of their ascarids. Spindler and Zimmerman (1944) placed three worm free groups of shoats on lots heavily infested with eggs or larvae of *Ascaris, Oesophagostomum, Trichuris, Ascarops, Physocephalus, Metastrongylus* and *Choerostrongylus*. One group of pigs was fed grain and water each morning and skim milk each evening, another group at intervals of three weeks in test one and two weeks in test two received skim milk exclusively for a period of three days, and the last group was given grain only. After three months the numbers of parasites harbored by the milk fed shoats were much less or entirely absent in comparison to the group maintained on grain. Leese (1943) reported beneficial results following administration of cow's milk to colts infected with strongyles.

The investigation herein reported was performed to note the effect of milk in a diet upon the susceptibility of a host toward *Trichinella spiralis*.

MATERIALS AND METHODS

The animals used in this investigation were white mice. Two weeks after receiving their respective rations each animal was parasitized with a small portion of rat diaphragm containing 100±8 microscopically counted *Trichinella spiralis* cysts. The diet of the experimental groups of mice was a commercially prepared rat ration supplemented with whole milk; the control animals received the commercially prepared ration and water.

The numbers of adults and muscular larvae harbored served as criteria of host resistance toward the trichinae. The adult trichinae were obtained six days after parasitism by removing the small intestine and caecum of each mouse. These organs were slit, cut into two inch lengths and refrigerated in a one percent solution of sodium hydroxide. After the mucus had dissolved, the adults were counted directly with a dissecting microscope. The larvae were obtained four weeks after parasitism by digesting the finely chopped, eviscerated, skinned carcass at 37° C. in 25 cc artificial gastric juice per gram of flesh.

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After digestion was completed the gastric juice was highly diluted with water and set aside several hours or until the larvae had settled to the bottom. The supernatant fluid was then drawn off. This process was repeated until the liquid containing the larvae was clear. The larvae were then concentrated and counted in five separate 0.5 cc samples on a hookworm egg counting slide. The total number of larvae harbored per mouse was then determined by multiplying the mean of these counts by twice the total number of cubic centimeters of solution containing the larvae.

EXPERIMENTAL DATA

The results presented here were taken from mice grouped into four experiments. The animals of each experiment were approximately 120 days old when trichinized with 100 ± 8 cysts.

Results obtained from adult T. spiralis *in mice.* Six days after parasitism the 10 mice on the milk diet in the first experiment (Table 1) were found to harbor a total of 250 adult worms. This was 25 trichinae per mouse. The 10 animals in the water group of the same experiment harbored a total of 240 trichinae or an average of 24 adult worms per mouse. In the second experiment (Table 1) the 13 mice of the milk group yielded a total of 404 or an average of 31.1 adult trichinae, while the 12 mice given water in place of milk yielded a total of 337 or an average of 28.1 adult worms.

When the means of the adult trichinae harbored by the milk fed mice whose diet was supplemented with whole milk were compared with the means of the adult worm numbers harbored by the mice which received water in place of milk in each of the experiments (Table 1), it was indicated that the milk had no effect upon the mice toward adult *T. spiralis* infections.

Results obtained from T. spiralis *larvae harbored in mice.* Two experiments of two groups each were performed on white mice in which the numbers of larvae harbored served as the criterion of the value of milk supplementing a diet. The animals were autopsied 28 days after parasitism with 100 ± 8 *T. spiralis* cysts.

In the first experiment (Table 2) it was found that a total of 45,322 larvae were harbored by the 17 mice receiving the milk diet. The same number of mice fed a diet in which milk was replaced by water had a total of 68,028 larvae. The average number of larvae harbored by the milk fed mice was 2,666 while the mice without milk harbored an average of 4,001 larvae. The wide difference in the average numbers of larvae harbored by the two groups of mice indicated a marked limitation of muscle invasion by trichina larvae in mice whose diet included milk.

Table 2 shows the results of a second experiment performed on two groups of animals. The 13 animals of the milk group harbored a total of 7,007 larvae while the 13 mice in the group without milk yielded 98,709 larvae. The musculature of the milk group of mice with an average of 539 larvae was much less infected by trichina larvae than the water group of mice with an average of 7,593 larvae.

DISCUSSION

It has been found by several investigators that the numbers of intestinal worms of a host were greatly reduced by the presence of milk in the diet (Ackert and Beach, 1933; Ackert and Riedel, 1946; Spindler, Zimmerman and Hill, 1944 and

TABLE 1.—*Comparison of the mean numbers of adult* T. spiralis *harbored by the two different diet groups of mice autopsied six days after infection with 100 ± 8 cysts.*

Diet group	Number and sex of hosts	Range in number of adults harbored	Total number of adults harbored	Average number of adults per mouse
	Experiment 1			
Milk	5F 5M	12–31	250	25
Water	5F 5M	4–37	240	24
	Experiment 2			
Milk	7F 6M	4–64	404	31.1
Water	6F 6M	6–40	337	28.1

Spindler and Zimmerman, 1944). In the present study on mice it was found that milk as a supplement in a diet did not reduce the average number of adult *T. spiralis* harbored. This may be explained on the basis that in the present study the period of parasitism was six days while the period of parasitism on fowl ascarids was about three weeks in each of the studies by Ackert and Beach (1933) and Ackert and Riedel (1946) and three months in the study of pig parasites by Spindler and Zimmerman (1944). Judging from their results the period of parasitism in the present investigation should have been longer than six days. Spindler, Zimmerman and Hill (1944) did find that a short 3–5 day milk treatment greatly reduced the number of parasites harbored by the pigs in their experiments, but they used an all skim milk diet while in the present study the diet was only partly of milk.

Although milk did not affect the average adult worm numbers harbored, the means of the larva numbers harbored by the mice receiving the milk were much lower than those of the mice without milk. This showed the milk had no affect upon the adult trichinae deeply located in the intestinal mucosa, but that it did eliminate many of the newly deposited larvae which found their way into the intestinal lumen before distributed throughout the host body by way of the intestinal lymphatics and mesenteric venules.

The results of the numbers of larvae harbored by the mice of the milk groups

TABLE 2.—*Showing the numbers of larvae obtained from the mice of the diet groups in each of the two experiments 28 days after administering 100 ± 8* T. spiralis *cysts.*

Experiment I				Experiment II			
Sex of mouse	Larvae harbored by milk group	Sex of mouse	Larvae harbored by water group	Sex of mouse	Larvae harbored by milk group	Sex of mouse	Larvae harbored by water group
F	60	M	900	M	0	F	1200
M	120	M	1052	M	40	M	4800
F	217	F	1145	M	250	M	6400
M	232	F	1240	F	258	M	6600
F	280	F	1350	M	300	F	7041
M	296	M	1654	F	360	F	7058
M	336	M	1940	F	389	F	8400
F	340	M	2067	F	405	F	8400
M	436	F	2090	F	420	F	8800
M	532	F	2262	M	520	M	9000
F	1000	M	2328	M	560	F	9010
F	1298	M	2400	F	905	M	9400
F	2528	M	3020	F	2600	M	12600
M	5006	M	4402				
F	5961	F	6328				
M	8080	F	7400				
F	18600	F	26450				
Total	45322		68028	Total	7007		98709
Average	2666		4001	Average	539		7593

(Table 2) show great variation in comparison to the groups fed a ration supplemented with water. This was attributed to the feeding habits of the animals. It was noted that some animals consumed large quantities of milk and less of the grain ration while others consumed less milk and more of the grain ration. The larger quantity of milk consumed in the diet probably resulted in fewer trichina larvae entering the musculature of the mouse.

SUMMARY

The effect of milk upon resistance of mice toward *Trichinella spiralis* was studied. On the sixth day after parasitism with 100 ± 8 cysts the average numbers of adult parasites harbored indicated that mice fed a commercial ration supplemented with whole milk harbored about the same numbers of adult parasites as mice fed the same ration supplemented with water. The smaller average numbers of larvae harbored 28 days after infection showed that whole milk supplementing a commercial, rat ration prevented many of the larvae from entering the general musculature of the mice.

LITERATURE CITED

ACKERT, J. E. and BEACH, T. D. 1933 Resistance of chickens to the nematode, *Ascardia lineata*, affected by dietary supplements. Trans. Amer. Micros. Soc. **52**: 51–58.

————and RIEDEL, B. B. 1946 Milk as a factor in fowl ascarid control. J. Parasit. **32**: (suppl.) : 15.

LEESE, A. S. 1943 Parasitic aneurism in foal. Vet. Jour. **99**: 276.

PORTER, D. A. 1935 Studies on the effect of milk diet on the resistance of rats to *Nippostrongylus muris*. Amer. J. Hyg. **22**: 467–474.

SPINDLER, L. A. and ZIMMERMAN, H. E. JR. 1944 Effect of skim milk on the growth and acquisition of parasites by pigs under conditions of constant exposure to infection. Proc. Helm. Soc. Washington. **11**: 49–54.

————ZIMMERMAN, H. E. Jr. and HILL, C. H. 1944 Preliminary observations of the control of worm parasites in swine by the use of skim milk. Proc. Helm. Soc. Washington. **11**: 9–12.

A SURVEY OF HELMINTH AND PROTOZOAN INCIDENCE IN MAN AND DOGS AT FORT CHIPEWYAN, ALBERTA.

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DIPHYLLOBOTHRIUM LATUM IN MAN

This survey of parasites centered around the incidence of the tapeworm *Diphyllobothrium latum* (Linn.) in man. Its presence was expected in the far north since the plerocercoid larvae were already reported from pike and pickerel by Rawson (personal communication). The author went to Fort Chipewyan for the summer of 1945 with other projects in mind, but finding evidence of human tapeworm infection a microscope was brought in by air and the survey initiated.

Fort Chipewyan is at the west end of Lake Athabasca, approximately 370 air-line miles north of Edmonton, Alberta, 58°45′ N and 111° W.

METHODS

Stool samples were obtained from as many persons as possible, regardless of age or race, who had lived at least a year in the north. The children at the Mission School formed the majority of the examinees since they were readily accessible and made no objection. Examination was by simple smear method using a low power of the microscope; ova of *D. latum* are easily recognised and usually numerous if present at all.

RESULTS

Of 140 persons examined 16 were positive (11.4%). But the 140 should not be regarded as a fair cross-section of the population of a northern post, since the very people most likely to be infected are the least co-operative.

FACTORS AFFECTING INCIDENCE

The population of all the vast north country is typified in Fort Chipewyan: a mixture of pure-blooded Indians (Chipewyans and Crees), half-breeds and a handful of whites. The natives are essentially nomadic; they have a cabin for the winter on the trap line, camp along the lake-shore and rivers during the fishing season, and live in a house or camp at the settlement in off seasons. However the establishment of a public school in Fort Chipewyan is tending to hold at least the mothers and children in "town" for the winter, whereas the Roman Catholic Mission School is residential thus leaving the parents free to follow their wandering life. These matters have a very definite bearing on the tapeworm incidence, since infection occurs chiefly under makeshift cooking conditions. A party travelling by boat will build a fire on shore, filet some fish and prop the filets on sticks before the fire; when warmed on both sides they are considered cooked. The popular taste is decidedly towards the under-done: the Indians relish a stew of meat to which raw shredded fish is added, while an old Russian trapper with an Indian wife told the author that they liked fish boiled "as long as you boil an egg."

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It was possible to examine only 16 persons who were known to lead the nomadic life and 11 were positive for *D. latum,* or 69%, a very different figure from that given above for the general population. In two families where only the father or father and eldest son trapped, only these were infected. The lamentably small number of such people examined is due to the indifference or active suspicion encountered, which no amount of explanation and pleading could overcome. Everyone who has lived in the far north knows the maddening trait of the native of agreeing to anything rather than refuse a white man, at the same time having no intention of carrying out the promise unless it suited him. Constipation was another complication: the natives are notoriously constipated and even a willing patient could sometimes not produce a sample for several days, by which time he had often moved elsewhere.

At the Mission School 49 children remained through the summer and only two were positive. When 31 others came in the fall after spending the summer with their parents in camps and settlements a much higher incidence was anticipated, but three positives was not a greatly higher percentage.

DISTRIBUTION

While the main survey was conducted at Fort Chipewyan, Alberta, a brief visit was made to the hospital at Fort Smith just within the North West Territories. Of the thirteen patients examined there, several of whom might reasonably have been expected to harbor tapeworms, only two girls were positive and these had both come from Fort Chipewyan for hospitalisation. However, the doctor in charge stated that he frequently had cases locally.

Of distribution further north there is definite evidence in the form of plerocercoids taken in fish at Great Slave Lake by Rawson. The author has been assured by several independent witnesses that doctors as far north as Aklavik at the mouth of the Mackenzie River encounter many cases in Indians and Esquimeaux. This cannot be taken as positive proof, but it does indicate that *Diphyllobothrium latum* is probably distributed all through the vast north country. To the south it extends to Montreal Lake and Waskesiu Lake in Saskatchewan and Lake Winnipeg in Manitoba. It is probably too late to determine whether this tapeworm was originally brought by immigrants from northern Europe and spread by man, dog and fish, or whether the parasite is indigenous in North America.

PATHOGENICITY

Enquiry of all infected persons failed to disclose any symptoms beyond occasional vague abdominal discomfort and slight nausea, accompanied by the usual disturbance of appetite associated with tapeworm infection: a large meal cannot be taken, but the patient is hungry an hour or so later. One old trapper claimed that after successful treatment he could do twice the work without tiring, but psychology may have had something to do with this. Only one positive suspected his infection; all the others seemed quite unaware. No instances of anaemia due to fish tapeworm infection were encountered and Wherrett (1945) lists anaemias in the North West Territories as less than 0.1 per 100,000.

TREATMENT

It was possible to treat eight of the infected persons. The standard method was employed, namely a purgative of 1 oz. Castor Oil at bed time, Male Fern in the morning without breakfast, three or four capsules according to age in two doses half an hour apart, followed in two hours by a purgative of Epsom Salts. In two instances the tapeworm yielded to the castor oil alone and was passed in the night. No instances of toxicity to male fern were encountered, but one boy of nine years proved resistant and no alternative vermifuge was available to try on him. The others all produced apparently complete worms although the scolex was recovered only in the Mission cases where sanitary conditions made successful examination possible. One Indian girl of 10 years age at the Mission produced two complete tapeworms 12 ft. and 23 ft. long after treatment on August 3 and yet examination on September 10 revealed ova still present. The author was unable to stay for the result of further treatment. Another child of 11 years showed very few eggs, all empty, in the stool; this case was left alone, as treatment of a dog with similar evidence had produced a tapeworm in the last stages of disintegration.

OTHER PARASITES OF MAN

No cases of infection with *Taenia solium* or *T. saginata* were found, nor were they expected since practically all pork and beef must be brought in from outside where it would be government-inspected.

PINWORM, *Enterobius vermicularis* (Linn.)

Faecal examination will not reveal the presence of pinworms and the accepted diagnostic procedure employing an anal swab was not generally feasible under northern conditions, so no figures of incidence can be given. However, many persons of all ages up to 65 years complained of sleepless nights due to anal pruritus attributable to pinworm. Whether the incidence equals that of *D. latum* or not, the author considers *Enterobius* the more harmful of the two to human welfare in the north.

No Trematode or *Ascaris* ova were seen.

PROTOZOA

After checking for tapeworm eggs, search was made for cysts and trophozoites of intestinal protozoa, using direct smear examination only. With few exceptions only one examination was made of each case, so the incidence recorded below is undoubtedly lower than the actual occurrence.

Protozoan and Helminthic Parasites in Man (140 Persons Examined)

Species	Number positive	Percentage
Endamoeba histolytica	4	2.9
Endamoeba coli	31	22.0
Iodamoeba bütschlii	10	7.1
Endolimax nana	5	3.5
Dientamoeba fragilis	4	2.9
Giardia lamblia	19	13.6
Diphyllobothrium latum	16	11.4
Taenia spp.	nil	...
Ascaris	nil	...

Having no concentration or staining checks on the direct iodine smear observations, the author reports with some hesitancy the incidence of *E. histolytica* so much

farther north than any previous records. It was the small-cyst race encountered occasionally in Saskatoon (Miller 1939), non-pathogenic, and was in Indians and half-breeds, not whites who might have been infected outside.

Several familial infections were noted with *Giardia;* otherwise the occurrence of all protozoa was distributed indiscriminately among the native and white population.

DIPHYLLOBOTHRIUM LATUM IN DOGS

Considering the common custom of feeding raw fish to dogs, infection was not as high as might be expected. Of 88 dogs examined 38 or 47.5% were positive and only a few of these showed large numbers of ova. The survey covered dogs kept around the settlement, loose and confined, and many more in "dog camps" where they are kept for the owners during the summer. The latter were fed boiled fish and "dog foods" made of meal and dried meat, but even then 34% were positive, probably as a hangover from winter feeding of frozen fish on the trail. It was noticeable that most of the positives showed comparatively few ova, and some produced only empty eggs which proved upon treatment to come from almost dead worms.

Treatment. Arecolin hydrobromide was used in the few cases treated. It was found difficult to pass the tablet through the stomach without vomiting, even in a gelatin capsule. The Brother in charge of the Mission dogs provided a solution: he administered a bowl of bouillon immediately after the piece of fish or meat containing the tablet in a gelatin capsule and he never lost the drug by vomiting.

OTHER PARASITES IN DOGS

In ten cases, the ova of the dog hookworm, *Ancylostoma caninum,* were recognised, and other nematode eggs occurred sparingly. In one faecal sample a single cyst of the Coccidian *Isospora,* probably *bigemina,* was seen. But no Trematode ova were encountered although the Liver Fluke of Dogs, *Parametorchis manitobensis,* occurs in northern Saskatchewan (Montreal Lake) and might be expected farther north and west.

Literature Cited

Miller, M. J. 1939 The intestinal protozoa of man in Saskatchewan. Can. Med. Ass. Jour. **41**: 120–124.

Wherrett, G. J. 1945 Survey of health condition and medical and hospital services in the North West Territories. Can. J. Econ. and Pol. Sci. **11**: 49–60.

THE ROLE OF POTASSIUM AS CAUSE OF DEATH IN EXPERIMENTAL TRYPANOSOMIASIS[1]

George J. Scheff[2] and Jonathan S. Thatcher

The ultimate cause of death in experimental trypanosomiasis, like that of many other diseases, is still a matter of controversy. Among the various earlier theories proposed from time to time, the more prevalent ones were: (a) mechanical obstruction (Andrews, Johnson, and Dormal, 1930); (b) formation of trypanotoxin (Schilling and Rondoni, 1913; Martin and Darré, 1914; Reichenow, 1921; Regendanz and Tropp, 1927, 1929; Kligler, Geiger, and Comaroff, 1929); (c) acidosis (Andrews, Johnson, and Dormal, 1930; Nierenstein, 1908; Scheff, 1928, 1932; Kligler and Geiger, 1928; Linton, 1929, 1930; Linton and Poindexter, 1931; von Brand, Regendanz, and Weise, 1932); (d) hypoglycemia (Regendanz and Tropp, 1927, 1929; Scheff, 1928, 1932; Scheff and Csillag, 1936; Linton, 1929, 1930; Linton and Poindexter, 1931; Schern, 1926, 1928, 1930; von Fenyvessy, 1926; Dubois, 1926, 1928; Dubois and Bouckaert, 1927; Bruynoghe, Dubois, and Bouckaert, 1927; Savino, 1927; Cordier, 1927; Zotta and Radacovici, 1929; Angolotti and Carda, 1929; Locatelli, 1930; von Brand and Regendanz, 1931; Wormall, 1932; von Brand, 1938; Hoppe and Chapman, 1947); and (e) asphyxiation (Andrews, Johnson, and Dormal, 1930; Kligler, Geiger, and Comaroff, 1929; Scheff, 1928, 1932); Scheff and Rabati, 1938. To all of these hypotheses the objection was commonly raised that they are not primary but secondary causes. Zwemer and Culbertson (1939) introduced a new explanation. These workers observed a gradual increase in serum potassium levels of rats concomitant with progressive cytolysis during the course of trypanosome infection and attributed the ensuing death of the animals to potassium poisoning. More recently Ikejiani (1947) confirmed Zwemer and Culbertson's principal findings; however, arrived at the conclusion that the elevation of potassium in the plasma is also no more than a terminal effect. This would leave the primary cause still unsolved.

Since one of us (Thatcher, and Radike 1947) has previously shown that a systemic tolerance to potassium could be developed in rats, an opportunity was presented to study this question anew from a somewhat different angle. It was reasoned that if potassium poisoning plays a decisive rôle in the outcome of the trypanosome infection, animals which were tolerant to potassium ion, should demonstrate an increased resistance to the infection, as measured by the survival time, when compared with non-tolerant control animals.

It was with this specific object in mind that these experiments were planned.

METHODS

Two series of experiments were conducted, employing a total of 166 male albino rats of 160–200 grams of weight. The animals were subdivided into groups large

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enough to be of statistical significance. Both experimental and control animals were kept under the same conditions, and all animals were supplied with food and water *ad libitum*.

Tolerance to the potassium ion was developed according to the method described by Thatcher and Radike (1947) : a 1.35 mol (10%) KCl solution was administered by stomach tube four times a day at four-hour intervals (8 and 12 a.m., 4 and 8 p.m.). An initial dose of 10 cc. per kg. of body weight was given and the size of the dose increased by 5 cc. per kg. every fourth day until a dosage level of 25 cc. per kg. was reached. A total of 12 days was required for the adaptation procedure.

The level of potassium tolerance existing at any given time was determined by the administration of four 25 cc. per kg. doses of the above 10% KCl solution at four-hour intervals to both the experimental and control animals. The length of survival of the experimental as compared with the control animals served as an index of the degree of tolerance achieved (960 minutes taken as full tolerance). Further details are given in the above-mentioned paper.

A strain of *Trypanosoma equiperdum* which had been carried in rats was employed for infection. The suspension for infection was prepared from the citrated blood of the "seed" animal by fractional centrifugation. The plasma containing the trypanosomes was diluted with isotonic glucose-saline solution in such manner that the injected fluid (1.0 cc.) contained about ten million parasites per rat. The animals were infected intraperitoneally. The trypanosome counts were made from blood smears taken from the tail, and reported in terms of the number of trypanosomes per thousand of erythrocytes.

RESULTS

Our findings have been derived from two series of experiments (A and B). As will be seen, Table I contains the "supporting" data illustrating the level of potassium tolerance during the critical periods of the experiments; and Table II shows the principal results demonstrating the survival of the potassium tolerant and non-tolerant animals after infection.

In the first set of experiments (A) 69 rats were divided into four groups and studied as follows: Group I and IIa (consisting of 15 rats each) were simultaneously adapted and tested for their attained tolerance to potassium ion as described above. It can be seen from Table 1 that both groups of animals were completetly tolerant to the massive doses of potassium administered in the test procedure. Groups III and IV (consisting of 20 and 19 rats respectively) remained untreated and served as normal, potassium non-tolerant controls.

The day after the potassium adaptation was completed, groups I and III (one potassium tolerant and one non-tolerant group) were infected with trypanosomes. All animals were kept under constant observation and the survival of each rat was measured in hours from the time of infection. As is shown in Table II, there was no significant difference in the survival time of the tolerant and non-tolerant animals (P value of Fisher, greater than 0.05).

Since it was known from the previous work (Thatcher and Radike, 1947) that the potassium tolerance gradually declines after the adaptation procedure is discontinued, it seemed necessary to ascertain the residual potassium tolerance effective at the termination of the experiment. To this end, group IIb, which had been tested

TABLE 1.—*Test of potassium tolerance*

Series	Group	No. of rats	Survival					
			1st dose (min.)*	2nd dose (min.)*	3rd dose (min.)*	4th dose (min.)*	Total (min.)**	Es***
A	I	15	240	240	240	240	960	No variation
	IIa	15	240	240	240	240	960	No variation
	IIb	15	223	146	45	26	431	± 60.8
	IV	19	195	47	2	...	243	± 28.5
B	V	15	240	240	240	240	960	No variation
	VI	15	240	240	240	240	960	No variation
	VII	15	172	131	128	128	559	± 116.0
	IX	13	209	41	1	...	251	± 32.0
	X	24	178	68	3	...	249	± 33.9

* Mean survival time in minutes following each test dose.
** Mean total survival time in minutes.
*** Standard error.
Group I—Tested one day following adaptation procedure.
Group IIa—Tested one day following adaptation procedure.
Group IIb—Animals of Group IIa tested for residual tolerance 7 days after first test.
Group IV—Non-adapted rats tested simultaneously with Group IIb.
Group V—Tested one day following adaptation. Infected with trypanosomes two days before test.
Group VI—Tested one day following adaptation simultaneously with Group V. Not infected with trypanosomes.
Group VII—Tested for residual tolerance at end of experiment.
Group IX—Non-adapted rats infected with trypanosomes 2 days before test.
Group X—Non-adapted, non-infected rats.

once before (simultaneously with group I) but carried without any further treatment, was subjected to a final potassium tolerance test, along with control group IV, on the day following the death of all trypanosome infected rats (i.e. 6 days after the time of infection and 7 days following the first potassium tolerance test). As can be seen in Table I, the potassium adapted rats had retained a highly significant degree of potassium tolerance as compared with the control animals (P value of Fisher, less than 0.01).

In the second set of experiments (B) a total of 97 rats was employed. These were divided into six different groups. Groups V, VI, and VII (15 rats each) consisted of the potassium adapted animals; while groups VIII, IX, and X (composed of 15, 13 and 24 rats respectively) were made up of normal, potassium non-tolerant animals.

The experiment was so designed that the entire span of the infection (in average, 106 hours) would fall more closely within the limits of the maximal tolerance of the potassium adapted animals. Hence, group V (tolerant) and groups VIII and IX (non-tolerant) were infected with trypanosomes two days before the maximal potassium tolerance was tested.

TABLE 2.—*Effect of potassium tolerance upon the development and course of trypanosome infection*

Series	Group	No. of rats	Trypanosome count**	Es***	Survival (hours)*	Es
A	I	15	..	....	78	± 4.2
	III	20	..	....	84	± 2.7
B	V	15	25	± 3.6	108	± 3.0
	VIII	15	27	± 3.6	105	± 1.5

* Survival in hours following trypanosome infection.
** Trypanosomes per 1000 erythrocytes 77 hours after inoculation.
*** Standard error.
Group I—Potassium tolerant rats infected with trypanosomes one day following test for maximal tolerance.
Group III—Non-tolerant rats infected with trypanosomes simultaneously with Group I.
Group V—Potassium tolerant rats infected with trypanosomes two days before test for maximal tolerance.
Group VIII—Non-tolerant rats infected with trypanosomes simultaneously with Group V.

As in the first experiment, the length of survival of the trypanosome-infected, potassium-tolerant rats (group V) was compared with that of the trypanosome-infected non-tolerant rats (group VIII). Trypanosome counts were also made during the course of infection in both of them in order to see whether or not there was any change in the progression of the disease. The maximal tolerance to potassium was tested at the expected peak in the potassium-adapted group VI, and compared with the adapted and non-adapted infected groups V and IX respectively. The residual tolerance was ascertained at the end of the experiment in the potassium-adapted group VII and compared with control group X (i.e. approximately 6 days after the time of infection, but only 4 days following the first tolerance test).

As can be seen in table II, there was no significant difference in the survival of the trypanosome-infected, potassium-tolerant group V and the infected non-tolerant group VIII. (P value of Fisher, greater than 0.05). Moreover, the mean trypanosome counts 77 hours after inoculation (ca. 29 hours before death) in both the tolerant and non-tolerant groups were in very close agreement. Hence, the multiplication rate of the trypanosomes must have been essentially the same in both groups of animals. Comparison of group V with group VI in table I shows that the presence of a two-day old trypanosome infection has no influence upon the development of a tolerance to potassium. Furthermore, inspection of groups VII and X in table I shows that a highly significant degree of residual tolerance was present at the end of the experiment (P value of Fisher, less than 0.01).

It should also be noted in table I that there was no significant difference in the resistance of groups IX and X to potassium (P value of Fisher, greater than 0.05). This shows that a two-day old trypanosome infection had no influence upon the natural resistance of rats to the potassium ion.

DISCUSSION

Our approach to the problem brought certain facts to light which make it very unlikely that potassium is the primary lethal factor involved. The results of both experiments indicate that the tolerant groups attained essentially the same degree of resistance to potassium as Thatcher and Radike have reported on much larger groups of animals. In spite of this tolerance there was no significant difference in the survival time between the tolerant and the non-tolerant groups. We have further shown: that potassium administration *per se* had no influence on the development of the infection; that trypanosome infection of two days duration did not affect the natural resistance of the animals to potassium ion; and also that the tolerant animals survived massive doses of potassium administration, much higher than would be expected to occur in the terminal phases of the infection. The increased potassium level in the blood, which has been reported by others, is demonstrable only within the last 15–20 hours of infection. This would indicate that the potassium was released from the tissues or blood elements at a time when irreversible pathological changes have already occurred in the animals. But no matter what the final mechanism might be, the mere fact that the death rate within the potassium tolerant and non-tolerant animals was the same, is strong enough evidence in support of the view that the increased potassium level is a secondary terminal effect only.

SUMMARY

The survival time after trypanosome infection was found to be the same in the potassium tolerant and non-tolerant animals, suggesting that the potassium is not the causative agent of death, as recently advocated.

REFERENCES

ANDREWS, J., JOHNSON, C. M., AND DORMAL, V. J. 1930 Lethal factors in experimental infections with *Trypanosoma equiperdum* in rats. Am. J. Hyg. **12**: 381–400.

ANGOLOTTI, E. AND CARDA, P. 1929 La glucemia en la trypanosomiasis éxpèrimental del cobaya. Med. Paises Calidos **2**: 468.

VON BRAND, TH. 1938 Metabolism of pathogenic trypanosomes and the carbohydrate metabolism of their hosts. Quart. Rev. Biol. **13**: 41–50.

——— AND REGENDANZ, P. 1931 Über Störungen des Kohlehydratstoffwechsels bei der Trypanosomiasis des Kaninchens. Biochem. Zeitsch. **242**: 451.

———, ———, AND WEISE, W. 1932 Der Milchsäuregehalt und die Alkalireserve des Blutes bei experimentellen Trypanosomeninfectionen. Centr. Bakt. Parasit. 1 Abt. Orig. **125**: 461–468.

BRUYNOGHE, M., DUBOIS, A., AND BOUCKAERT, J. P. 1927 Le sucre du sang au cours des trypanosomiases expérimentales. Bull. Acad. Roy. Med. Belge **5**: 142.

CORDIER, G. 1927 Étude de la glycémie et action du serum glucose et de l'insuline dans quelques cas de trypanosomiase expérimentale. C. R. Soc. Biol. **96**: 971.

DUBOIS, A. 1926 Le phénomène de K. Schern dans les trypanosomiases. C. R. Soc. Biol. **95**: 1130–1133.

———1928 Mort par hypoglycémie dans les trypanosomiases aiguës. C. R. Soc. Biol. **99**: 656.

——— AND BOUCKAERT, J. P. 1927 L'hypoglycémie au cours des trypanosomiases expérimentale à *Trypanosoma brucei*. C. R. Soc. Biol. **96**: 431.

VON FENYVESSY, B. 1926 Über die Bedeutung des Stoffwechsels der Parasiten für das Wirtstier bei der Trypanosomeninfektion. Biochem. Zeitsch. **173**: 289–297.

HOPPE, J. O. AND CHAPMAN, C. W. 1947 Rôle of glucose in acute parasitemic death of the rat infected with *Trypanosoma equiperdum*. J. Parasit. **33**: 509–516.

IKEJIANI, O. 1947 Studies in trypanosomiasis. II. The serum potassium levels of rats during infection with *Trypanosoma lewisi, Trypanosoma brucei,* and *Trypanosoma equiperdum*. III. The plasma, whole blood, and erythrocyte potassium of rats during the course of infection with *Trypanosoma brucei* and *Trypanosoma equiperdum*. J. Parasit. **32**: 374–382.

KLIGLER, I. J. AND GEIGER, A. 1928 Lactic acid content of blood of trypanosome-infected rats. Proc. Soc. Exp. Biol. and Med. **26**: 229.

———, ———, AND COMAROFF, R. 1929 Susceptibility and resistance to trypanosome infection. VII. Cause of injury and death in trypanosome-infected rats. Ann. Trop. Med. Parasit. **23**: 325–335.

LINTON, R. W. 1929 Blood sugar in infections with *Trypanosoma lewisi*. Ann. Trop. Med. Parasit. **23**: 307–313.

———1930 Blood chemistry of acute trypanosome infection. J. Exp. Med. **52**: 103–111, 695.

——— AND POINDEXTER, H. A. 1931 Artificial acidosis in *Trypanosoma lewisi* infections, and its bearing on the pathogenic action of *Trypanosoma equiperdum*. J. Exp. Med. **54**: 669–680.

LOCATELLI, P. 1930 Sur le metabolisme du glucose dans la trypanosomiase. C. R. Soc. Biol. **105**: 449.

MARTIN, L. AND DARRÉ, H. 1914 Documents sur la trypanosomiase humaine. Bull. Soc. Pathol. Exot. **7**: 711–716.

NIERENSTEIN, M. 1908 Observations on the acidity and alkalinity of the blood in trypanosome infections. Ann. Trop. Med. Parasit. **2**: 227–231.

REGENDANZ, P. AND TROPP, C. 1927 Das Verhalten des Blutzuckers und des Leberglykogens bei mit Trypanosomen infizierten Ratten. Arch. f. Schiffs und Trop.-Hyg. **31**: 376.

——— AND ———. 1929 Der Blutzucker bei Trypanosomeninfektionen. Arch. f. Schiffs und Trop.-Hyg. **33**: 242.

——— AND ——— 1929 Pathogenicity of *Trypanosoma lewisi* and blood sugar in infections with *Trypanosoma lewisi* and *Bartonella muris rattis*. Ann. Trop. Med. **23**: 523.

REICHENOW, E. 1921 Untersuchungen über das Verhalten von *Trypanosoma gambiense* im menschlichen Körper. Zeitsch. f. Hyg. und Infektionskr. **94**: 266–385.

SAVINO, E. 1927 Relation entre le nombre des trypanosomes et les variations glycémiques dans l'infection expérimentale par *Trypanosoma equiperdum*. C. R. Soc. Biol. **96**: 220.

SCHEFF, G. J. 1928 Über den intermediären Stoffwechsel der mit Trypanosomen infizierten Ratten. Biochem. Zeitsch. **200**: 309–330.

————1932 Über den intermediären Stoffwechsel der mit Trypanosomen infizierten Meerschweinchen. Biochem. Zeitsch. **248**: 168–180.

———— AND CSILLAG, Z. 1936 Über die Beziehungen einiger jodbindenden Substanzen (wie Glutathion, Ascorbinsäure) zum Kohlehydrathaushalt in der Trypanosomeninfektion. Arch. f. exp. Path. und Pharm. **183**: 467–477.

———— AND RABATI, F. 1938 Über die Einwirkung von Dinitrophenol auf den Energieumsatz und Sauerstoffverbrauch normaler und infizierter (Trypanosomen) Mäuse. Biochem. Zeitsch. **298**: 101–109.

SCHERN, K. 1926 The pathogeny of trypanosomiasis explained upon the basis of trypanosome sugar phenomena.—as given by Andrews et al 1930. Original probably in German—H.W.S. Berl. tierärztl. Wochensch. **42**: 665.

————1928 Über die Störung des Zuckerstoffwechsels bei Trypanosomiasen und Spirochätosen. Biochem. Zeitsch. **193**: 264–268.

————1930 Zur glykopriven Intoxikation bei der Trypanosomiase. Centr. Bakt. Parasit. 1 Abt. Orig. **119**: 297–302.

SCHILLING, I. AND RONDONI, P. 1913 Über Trypanosomentoxine und Immunität. Zeitsch. f. Immunitätsforsch. **18**: 651–665.

THATCHER, J. S. AND RADIKE, A. W. 1947 Tolerance to potassium intoxication in the albino rat. Am. J. Physiol. **151**: 138–146.

WORMALL, A. 1932 Carbohydrate metabolism in human trypanosomiasis. Biochem. J. **26**: 1777–1787.

ZOTTA, G. AND RADACOVICI, E. 1929 Contribution à l'étude du métabolisme du glucose sanguin dans la trypanosomiase expérimentale. Arch. Roum. Path. Exp. **2**: 55.

ZWEMER, R. L. AND CULBERTSON, J. T. 1939 The serum potassium level in *Trypanosoma equiperdum* infection in rats: the rôle of potassium in death from this infection. Am. J. Hyg., Sec. C **29**: 7–12.

AN ATTEMPT TO ADAPT *STRONGYLOIDES RATTI* TO THE MOUSE*

STERLING BRACKETT AND ALEXANDER BLIZNICK

As part of a program on the search for improved anthelmintics, infections with *Strongyloides ratti* (Sandground, 1925) were investigated as a possible tool for use in large-scale drug screening. As has been pointed out earlier (Brackett and Bliznick, in press), one of the prerequisites of an infection for such a purpose is its ready and uniform development in a small laboratory animal, preferably a mouse, because of the limited quantities of drug that are usually available for testing. Although Sandground (1925) was unsuccessful in passing *S. ratti* to mice, Sheldon (1937a) succeeded in establishing light infections in 4 of 12 mice inoculated subcutaneously with 500 larvae. Sheldon suggested the possibility of developing a "mouse" strain of this parasite by repeated passage through mice. This paper is a report on the results obtained after passing *S. ratti* infections 19 times through mice.

MATERIALS AND METHODS

The strain of *S. ratti* was originally obtained April 29, 1947 from Miss Evelyn Allen of the School of Hygiene and Public Health, The Johns Hopkins University, and maintained since then by passage in rats at about 2-week intervals. Larvae were cultured equally well in fecal pellets kept moist in a covered dish or in charcoal dilutions of the egg-bearing feces. The filariform larvae migrated from the fecal pellets out onto the margins of the moist filter paper under the feces. They were collected from the filter paper simply by immersing it in tap water. From charcoal cultures they were isolated by the standard Baerman technique. After counting the larvae by dilution techniques, they were permitted to sediment in a tube and the excess water was removed. A 0.1% agar solution was used to reconstitute the inoculum to give the desired number of larvae to be injected into each animal in 0.1 cc. The agar solution neither injured the larvae nor the hosts and served to keep the larvae in a uniform suspension during the time required to infect a series of animals. The larvae were injected subcutaneously, since Sheldon (1937b) has shown this route to give the most satisfactory results and our experience is corroboratory. Worm counts were made by removing the small intestine of test animals after several hours of fasting, storing them in the refrigerator for several hours, splitting them open and pressing them between two heavy glass plates and examining them with low magnification.

The mice used were obtained from Miss Irene Vanderwerken, Stamford, Conn., and the rats from Carworth Farms.

Results:

Preliminary trials indicated that the strain of *S. ratti* employed would develop into egg-bearing females in young (10–12 g) Vanderwerken mice. Cultures of the feces of these mice yielded filariform larvae which, in turn, were capable of developing into mature parasitic females in other young mice. For example, after one passage

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TABLE 1.—*The percentage of larvae of the "mouse" strain of* S. ratti *developing to maturity in young mice weighing 10–14 g*

Passage number	Approximate number larvae injected	Approximate number larvae per g of body wt.	Worm counts—4th to 7th day		Av. % development
			Individuals	Mean	
2	200	17	25, 3, 24, 8, 21, 0	13	6.5
9	450	37	13, 48, 111, 0, 57	46	10.0
	900	75	66, 226, 61, 33	96	10.5
10	500	42	31, 1, 16, 64, 79	38	7.6
	1000	85	8, 30, 12, 27	19	2.0
12	500	42	11, 57, 17, 143, 32, 31, 29	47	9.5
	1000	85	62, 0, 53, 34, 69, 0	36	3.6
	2000	170	31, 158, 81	90	4.5
13	600	50	3, 13, 41, 0, 2	12	2.0
	1200	100	124, 100, 11, 132	92	7.5
19	560	47	12, 82, 53, 99, 16, 25, 12, 72, 69, 37, 203	60	11.0
					Mean = 6.75

through mice, 200 filariform larvae were inoculated subcutaneously into 6 young mice which had worm counts on the 7th day, as follows: 25, 3, 24, 8, 21 and 0, or an average of 13 (passage 2, Table 1). The average percent development, thus, was 6.5. The strain was passed for 19 consecutive times through mice with tests made at intervals to determine whether the strain was becoming better adapted to this host as indicated by the percentage of inoculated larvae reaching maturity in the small intestine (Table 1). It is quite evident, without statistical analysis, that there was no trend toward greater adaptability. It is interesting, in view of the poor results Sandground and Sheldon had in infecting mice, that only 5 of 60 mice (8%) in Table 1 failed to harbor worms.

For sake of comparison, a number of young mice were infected by inoculations of larvae cultured from the strain being passed in rats (Table 2). The results as far as percent development and the percent of animals in which the infection became established can be seen to be about the same for both "strains" of the parasite. The overall average percent development for the "mouse" strain in mice was 6.7 as compared with 5.8 for the "rat" strain in mice.

The characteristics of the infections in rats of the strain of *S. ratti* used are shown in Table 3. Excluding experiment VI, in which an abnormally low percent development was encountered, possibly due to injury to the larvae in the inoculum, the mean percent development was 44 which is strikingly higher than that seen in mice. In

TABLE 2.—*The percentage of larvae of the "rat" strain of* S. ratti *developing to maturity in young mice weighing 10–14 g*

Exp. no.	Approximate number larvae per mouse	Approximate number larvae per g of body wt.	Worm counts—4th to 7th day		Av. % development
			Individuals	Mean	
I	300	25	1, 3, 0, 5, 5, 6, 48, 8, 12	9.8	3.0
II	325	25	13, 106, 68, 2, 3, 7, 21, 20, 14	28	8.5
III	450	37	0, 3, 16, 45, 7	14	3.0
I	600	50	91, 0, 50, 12, 10, 17, 40, 10, 98, 49, 21	36	6.0
I	900	75	26, 21, 8, 47, 34, 94 78, 87, 89	54	6.0
III	900	75	0, 5, 6, 20	8	0.9
II	1300	110	138, 127, 123, 259, 208	171	13.1
					Mean = 5.8

TABLE 3.—*The percentage of larvae of the "rat" strain of* S. ratti *developing to maturity in young rats weighing 35–40 g*

Exp. no.	Approximate number larvae per rat	Approximate number larvae per g of body wt.	Worm counts—5th day		Av. % development
			Individuals	Mean	
I	500	12	324, 165, 184	224	45
II	500	12	189, 227, 260, 327	251	50
III	275	7	161, 140, 169, 175	161	58
IV	250	6	112, 95, 97, 26	82	33
V	325	8	134, 143, 121, 62	115	35
VI	380	9	25, 17, 34, 41	29	7.6*
					Mean = 44

* This one low figure might have been due to some unkown factor reducing the viability of the larvae. The larval count had been double checked.

addition, the number of worms developing in individuals in a group seems to be less variable and infections developed in all 23 animals inoculated.

As has been indicated, mice weighing between 10 and 14 g (3 to 4 weeks of age) were used in the studies. This was done because of the probable greater susceptibility of younger animals as suggested by general information on age resistance to helminths and specifically by Sheldon's (1937c) report of the development in rats of an increased resistance to *S. ratti* with age. Sheldon found that rats 8, 12 and 17 months of age yielded from 1/3 to 1/2 as many worms from 1000 larvae as did rats 2 months old. The average percent development in 58 rats of about 8 weeks of age inoculated subcutaneously with 1000 larvae was 27.1 (Sheldon, 1937b), while in our experience a higher rate of development (44%) was obtained in rats 3 to 4 weeks of age (Table 3). Of course, part of this difference might be due to the difference in the strains of parasite or hosts used.

The relation of the age of the mouse host to its susceptibility to *S. ratti* is shown in Table 4. So far as these data are concerned, the mouse does not seem to become more resistant with age in respect to the average percent development of worms but may in respect to the percent of mice harboring worms. In only the very youngest group did all of the individuals harbor worms in the intestine. In this connection it must be kept in mind that the number of larvae inoculated was the same in all cases. Thus the youngest mice had the heaviest inoculation when calculated on the basis of larvae per gram of body weight; a point which might be related to the fact that they all become infected.

No attempt was made to determine whether the egg-output per female worm was increased with successive passage of *S. ratti* through mice.

DISCUSSION

While 19 passages of *S. ratti* through mice failed to increase its adaptability to this host, it is possible that more prolonged attempts might be successful. An

TABLE 4.—*Infection with the "rat" strain of* S. ratti *in mice of different ages—subcutaneous inoculation with 480 larvae*

Av. wt. of mice at time of infection	Approximate age of mice	No. mice inoculated	Mice with worms in intest.		Av. no. worms	Av. % development
			No.	%		
8.5	2.5 wks.	14	14	100	16	3.3
14.2	4 wks.	17	12	70	15	3.1
17.2	5 wks.	20	16	80	34	7.1
20.5	6 wks.	18	13	72	19	4.0

occasional mouse permitted the development of 25 to 37% of the larvae (Table 1, passages 9, 12 and 19), suggesting the possibility of developing, by selection and breeding, a strain of mice more susceptible to this infection. As a matter of fact, our results, as compared with those of Sandground (1925) and Sheldon (1937a), strongly suggest that we were already using a more susceptible strain of mice. Investigations of other strains might lead to the discovery of an even more susceptible strain.

The mouse, at least the strain used, is clearly a less favorable host than the rat for *S. ratti,* as shown by a lower percent development of larvae to adults, the greater proportion of individuals that failed to harbor any worms, and the greater degree of variability in individual worm counts.

Vanderwerken mice could be used for drug testing with infections with *S. ratti.* However, little if anything would be gained over using young rats of 35–40 g weight. The high degree of variability of worm counts in mice would make it necessary to use sufficiently larger numbers of experimental animals to more than compensate for the lower cost of mice and the smaller quantities of drug required for each treated mouse as compared with rats.

SUMMARY AND CONCLUSIONS

A strain of *S. ratti* was passed through young mice 19 times without increasing its adaptability to this host. About 6% of larvae inoculated into Vanderwerken mice weighing 10–14 g developed to mature worms and 80 to 90% of the mice inoculated harbored adult worms in the intestine. Infections with *S. ratti* in young mice could be used for drug screening purposes but would probably effect no economy over using young rats weighing 35–40 g because the high degree of variability of the infection in mice would require the use of two to three times as many mice as rats in any experiment.

REFERENCES

BRACKETT, STERLING AND BLIZNICK, ALEXANDER 1949 Screening large numbers of new chemical compounds for anthelmintic activity using infections with *Nippostrongylus muris* in mice. (In press.)

SANDGROUND, J. H. 1925 Speciation and specificity in the nematode genus *Strongyloides.* J. Parasit. **12:** 59–80.

SHELDON, A. J. 1937a Successful infection of mice with *Strongyloides ratti.* J. Parasit. **23:** 98.

————1937b Studies on routes of infection of rats with *Strongyloides ratti.* Am. J. Hyg. **26:** 358–373.

————1937c Age resistance in laboratory rats to infection with *Strongyloides ratti.* Am. J. Hyg. **26:** 355–357.

THE EFFECT OF ALCOHOL ON NATURAL AND ACQUIRED IMMUNITY OF MICE TO INFECTION WITH *TRICHINELLA SPIRALIS**

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That mice become partially immune to *Trichinella spiralis* as a result of prior infection was established by Culbertson (1942). The immunity acquired was shown to be directed particularly against the intestinal phase of the parasite, but the mechanism of this immunity was not learned. Since this investigator pointed out that mice have been used little in experimental trichiniasis, the present study on *T. spiralis* was planned in an attempt to gather additional information by determining the influence of alcohol on the natural and acquired immunity of this host. Earlier studies with this drug in mice have demonstrated that it interferes with the immune response to *Hymenolepis* and that the mechanism of the interference is related to the loss of vitamins (Larsh, 1945, 1946, 1947).

MATERIALS AND METHODS

The mice used were of the same strain used in the earlier work mentioned above. The mouse colony is housed in an air-conditioned room maintained at 75° F. and the animals are raised *Hymenolepis*-free. They are allowed food (Purina dog checkers) and water *ad libitum*. Mice for the present work were removed from this colony at five weeks and were divided into alcoholic and control groups on the basis of sex and approximate weight. They were continued on the above diet, and a record was kept throughout the experiment of food consumption and weight change. The techniques of these calculations appear in one of the earlier papers (Larsh, 1947).

The stock strain of *T. spiralis* is one that has been maintained in rats for a number of years.

Alcohol for dosage was diluted to 40 per cent. This strength of alcohol was determined from the earlier studies (Larsh, 1945, 1946). The drug was administered into the stomach by use of a blunted, slightly curved, 18 gauge needle and a 1 cc Tuberculin syringe. In the present series of experiments a sufficient amount of alcohol was given daily to produce complete narcosis. Animals that were not completely narcotized after one hour were given additional alcohol, the amount varying from 0.02 to 0.04 cc. The total daily injection did not exceed 0.39 cc. It is believed that this procedure is better than giving a fixed daily dose per animal, because the drug's effect is so variable in the individual mice and even in the same mouse from day to day. The controls were given at the same time an equal volume of normal saline.

In the present experiments involving continued alcohol treatment after one or more infections, the alcohol dosage was not renewed until seven days after infection to prevent possible interference with the establishment of the adult worms in the intestine. To avoid confusion in the discussion of experiments, animals given this

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continued alcohol treatment are referred to as re-alcoholics, whereas those given the drug only prior to the first infection are called x-alcoholics.

The following experimental methods were modified from those described by other workers. Such changes were made in an attempt to improve the accuracy of the methods and thus overcome, in part, the criticisms directed at many previous quantitative studies on *T. spiralis* (Rappaport, 1943, and others). Because in such studies the results depend in great measure upon the methods employed, it seems necessary to describe in detail the techniques used in the present work.

Larvae for infection were obtained from rats that had been infected not less than six weeks previously. The trichinous meat was minced and digested with constant agitation by means of a small electric stirrer at 37.5° C for one hour in artificial gastric juice consisting of 1 per cent hydrochloric acid and 0.7 per cent pepsin. Twenty-five cc of this solution were used per gram of meat. Following digestion, the mixture was strained through two thicknesses of gauze and the total volume of the filtrate was doubled with 0.8 per cent saline. The larvae were concentrated in a 50 cc centrifuge tube by a process involving centrifugation at 1800 r.p.m. for one minute (145 times gravity), removal of supernatant fluid by aspiration and the pooling of the sediment. Nutrient broth with gelatin was added to assist in retaining the larvae in suspension long enough for accurate counts (Culbertson, 1942). If the mixture was of too high viscosity, it was diluted with saline. Using the syringe and needle mentioned above, the material was mixed thoroughly and 0.1 cc samples removed for counting. The number of larvae was determined by successive counts and fixed at 300 per 0.1 cc. Infection of animals was always performed by the same person using this syringe and needle and forcing the larvae into the stomach. The mice were infected at 3:00 P.M. on the same day that the larvae were prepared.

The first step in the recovery of adult worms from infected mice was the removal of food eight hours before killing the animals to reduce the amount of semi-digested material in the small intestine. The animals were killed and the entire small intestine removed and placed in a three inch glass funnel. The intestine was then slit longitudinally and cut transversely into small pieces about one inch in length. The contents of the funnel were flushed with 175 cc of 0.05 per cent sodium hydroxide solution into a wide mouthed, screw cap jar of 500 cc capacity. Such jars were refrigerated overnight at 45° F. The following day each jar was shaken 100 times and the small pieces of intestine removed. Repeated examination of intestinal material so treated failed to disclose any adult worms still attached to or imbedded in the mucosa. Adult worms were concentrated by centrifugation as above until a total volume of approximately one cc was obtained. This entire sediment and fluid, including that used for rinsing, was examined and a count of all adults was made. A stereoscopic microscope was used with 2× objectives and 15× oculars. All the sediment and fluid were transferred by bulb pipette to a specially prepared glass slide 30 cm long, ten cm wide and two mm thick. The material to be examined was expelled from the pipette in longitudinal, parallel lines. Care was taken that the width of these lines was not greater than that of the microscopic field. Because of the length of this slide, it was helpful to use extension side arms on the stage to facilitate handling. Hand tallies were used for recording the numbers of worms found.

The methods of recovering and counting the larvae were as follows. Each mouse was skinned, eviscerated and thoroughly minced with scissors. It was then placed in the artificial gastric juice described above in an Ehrlenmeyer flask of suitable size. These flasks were placed in a large incubator at 37.5° C and left, with occasional agitation by hand, until digestion was complete except for bone—usually from eight to 12 hours. The mixture was strained through two thicknesses of gauze to remove the bone. The gauze was rinsed thoroughly and the larvae concentrated in graduated, pointed, 50 cc centrifuge tubes. The supernatant fluid after the final pooling and centrifugation was removed by aspiration to 5 cc total volume. This volume was increased to 10 cc with the nutrient broth-gelatin mixture. Using the same syringe and needle as described above for infecting, a uniform suspension of the larvae was obtained by the use of 15 compression strokes of the plunger. On the last stroke, 0.1 cc of the suspension was withdrawn from the center of the tube and streaked immediately on the long glass slide described above. The streak was diluted with several drops of tap water, care again being taken that the width of the streak did not exceed that of the microscopic field. A hand tally was used in recording the counts. A series of counts was made until three consecutive ones did not vary more than 20. The entire number of larvae per mouse was established from the average of three such counts multiplied by the dilution factor, 100×.

Counts of larvae were not made in animals dying within 30 days after infection, because of the possibility of error due to loss of immature forms in the digestion process (McCoy, 1934).

Statistical analysis of all of the data was performed by calculating the ratio of the observed difference to its standard error. Values greater than 2.00 were considered significant. Values of such magnitude indicate that the results might have occurred by chance in only five out of 100 similar experiments.

EXPERIMENTAL RESULTS

I. *Studies on the effect of alcohol on natural immunity*

EXPERIMENT 1.—*Effect of 20 daily doses of 40 per cent alcohol as shown by recovery of adult worms one week after a single infection.*—Experimental and control animals were matched according to age, weight and sex. After three days of alcoholization at low dosage (0.15 cc per day) to allow adjustment of the animals to the toxic effects of the drug, complete narcosis was produced in all of the alcoholic animals. Twenty-four hours after the last dose of alcohol, 230 larvae were given to the mice of both groups. No alcohol was administered after infection. One week after infection the animals were sacrificed and counts of adults were made by the method described above. The number obtained from each mouse is given in Table 1.

The high recovery of adult worms from mice of both the alcoholic group (average 130.6) and the control group (average 150.4) indicates little natural resistance to this parasite. There was apparently little difference in the immune response of the alcoholic and control mice, despite the fact that about 36 per cent of the former group were lost during treatment and debilitation was striking in those that survived. The difference in development (ratio of adults recovered to the number of larvae in the infecting dose: average for alcoholic mice, 0.57 and for controls, 0.65) must be

TABLE 1.—*The effect of alcohol on the numbers of adult* Trichinella *present one week after a single infection with 230 larvae*

Alcoholic mice			Control mice		
Mouse no.	No. adults recovered	Ratio to no. larvae given at infection	Mouse no.	No. adults recovered	Ratio to no. larvae given at infection
1	142	0.62	1	143	0.62
2	132	0.57	2	167	0.73
3	155	0.67	3	188	0.82
4	110	0.48	4	116	0.50
5	113	0.49	5	176	0.77
			6	143	0.62
			7	120	0.52
Average 130.6		0.57	Average 150.4		0.65

attributed to individual variation as analysis of the data shows the difference is not significant statistically.

EXPERIMENT 2.—*Effect of 34 daily doses of 40 per cent alcohol as shown by recovery of adults and larvae 30 days after a single infection.*—Experimental and control animals were matched as for experiment 1. Other conditions were similar except for prolongation of the period of alcohol dosage prior to infection in an attempt to produce greater debilitation. The infecting dose was 300 larvae given 24 hours after the final alcohol treatment. The animals were killed 30 days following infection to determine the persistence of adult worms and the numbers of larvae present. Results for the individual mice are shown in Table 2.

Although somewhat higher recovery of adults was obtained from the alcoholic group (average 1.6) than from controls (average 0.38), it appeared that the obvious debilitation of the former should have produced a more notable effect on resistance. The persistence of adults 30 days after infection is of interest since the period of adult persistence has previously been reported for mice as being 16 days (Gould, 1945). Statistical evaluation indicates no significant difference in the numbers of larvae recovered from the alcoholic mice and controls (average ratios to infecting dose 244.4 and 201.3, respectively).

II. *Studies on the effect of alcohol on acquired immunity*

In the following experiments, any dose of larvae administered in an attempt to produce immunity in the mice is designated as a stimulating infection, whereas the dose used in measuring the degree of immunity produced is designated as the challenging infection.

TABLE 2.—*The effect of alcohol on the numbers of adults and larvae present 30 days after a single infection with 300 larvae*

Alcoholic mice				Control mice			
Mouse no.	No. of adults in intestine	No. of larvae in muscles	Ratio to no. larvae in infecting dose	Mouse no.	No. of adults in intestine	No. of larvae in muscles	Ratio to no. larvae in infecting dose
1	1	43,600	145×	1	0	51,000	170×
2	0	67,000	223×	2	0	61,200	204×
3	3	104,500	348×	3	0	75,900	253×
4	1	53,100	177×	4	1	79,500	265×
5	3	98,600	329×	5	0	59,200	197×
				6	0	44,000	143×
				7	1	63,300	211×
				8	1	50,000	167×
Average 1.6		73,360	244.4×	Average 0.38		60,513	201.3×

EXPERIMENT 1.—*Effect of alcohol on immunity acquired from a single stimulating infection of 300 larvae.*—Conditions of this experiment were somewhat similar to those for studies on natural immunity. Twenty daily doses of 40 per cent alcohol were given to each of the alcoholic mice prior to infection. Twenty-four hours after the final alcohol dosage all of the alcoholic and control mice were given a stimulating infection with 300 larvae. After seven days the alcoholic treatment was renewed in half of the alcoholic group (re-alcoholics). This group received an additional 23 daily doses of alcohol. The other half of the alcoholic group (x-alcoholics) was not given this additional alcohol. At the end of 30 days following the stimulating infection, the immunity developed in the alcoholic and one-half of the control mice was challenged by an infection of 300 larvae. The other one-half of the control animals (three of each sex) was retained as a check on persistence of adult worms. Seven days after the challenging infection, all the animals were sacrificed and adults and larvae counted. The results, except those for the second control group, are shown in Table 3.

TABLE 3.—*Showing the numbers of adults and larvae in alcoholic and control mice given one stimulating infection of 300 larvae and killed one week after a challenging infection of 300 larvae*

No. mice	No. adults in intestine (average)	Average ratio to no. larvae in challenging infection	No. larvae in muscles (average)	Average ratio to no. larvae in stimulating infection
A. X-alcoholic mice				
4	113	0.38	53,450	178.2
B. Re-alcoholic mice				
4	141.5	0.47	42,500	141.7
C. Control mice given saline				
6	120	0.40	52,259	174.2

Apparently few adult worms persisted from the first infection, as there were only two found in the six controls given only the stimulating infection. The average number of larvae in the muscles of these six mice was 41,258 (ratio to infecting dose, 138). As for the remaining animals, there is no significant difference in the numbers of adults and larvae recovered from mice of the various groups. These analyses included a comparison between the x-alcoholics and controls (given both infections), the x-alcoholics and the re-alcoholics and the re-alcoholics and controls. In the six comparisons, the ratio of the observed difference to its standard error did not in any case exceed 1.21.

EXPERIMENT 2.—*Effect of alcohol on immunity acquired from three stimulating infections of 300 larvae each.*—A schedule was followed whereby all of the alcoholic mice received 28 doses of alcohol prior to the first infection. Thereafter, only the re-alcoholics were given the drug, which was administered on a staggered basis between infections. So that these time intervals may be presented, the following is an account of how the various groups were handled. The first stimulating infection of 300 larvae was given to all mice 24 hours after the alcohol treatment. Seven days later the alcoholic mice were divided into two groups, denoted as in the preceding experiment, x-alcoholic and re-alcoholic. The x-alcoholic group received

no further alcohol for the duration of the experiment. In the re-alcoholic group, however, alcohol was continued an additional 28 days. Twenty-four hours after this alcohol treatment all mice (experimentals and controls) were given the second stimulating infection of 300 larvae. Again, after the usual seven day period to permit adult attachment in the intestine, alcohol treatment was renewed in the re-alcoholic group. This time the re-alcoholics received only seven daily doses of alcohol. Twenty-four hours after the last dose of alcohol, all mice were given the third stimulating infection of 300 larvae. Seven days later alcohol treatment was renewed for the final period in the re-alcoholic group which again received seven daily doses of the drug. Twenty-four hours later, the immunity developed by the various mice was challenged by an infection of 300 larvae. The re-alcoholic group, therefore, received a total of 42 daily doses of 40 per cent alcohol in addition to the amount received by the x-alcoholics. Because the above work showed that few worms persist for 30 days, in the present case the animals were sacrificed two weeks after the challenging infection and counts were made of adults and larvae (table 4).

TABLE 4.—*Showing the numbers of adults and larvae in alcoholic and control mice given three stimulating infections of 300 larvae each and killed two weeks after a challenging infection of 300 larvae*

No. mice	No. adults in intestine (average)	Average ratio to no. larvae in challenging infection (300)	No. larvae in muscles (average)	Average ratio to no. larvae in three stimulating infections (900)
A. X-alcoholic mice				
4	32	0.11	87,025	96.7
B. Re-alcoholic mice				
4	52.5	0.18	144,500	160.6
C. Control mice given saline				
5	38	0.13	55,940	62.2

The difference in the numbers of adult worms found in the alcoholic and control mice is not significant. On the other hand, the data show that alcohol dosage coupled with reinfection under the experimental conditions resulted in higher recovery of larvae. Both the x-alcoholics and the re-alcoholics showed significantly greater numbers of larvae than did the controls, average ratios 96.7, 160.6, and 62.2. In addition, it is worth noting that the re-alcoholics harbored significantly greater numbers of larvae than the x-alcoholics. These ratios are obtained by dividing the number of larvae recovered by the number in the infections given at least 30 days prior to autopsy, in this case 900.

It is interesting that the numbers of larvae found in the controls of this experiment (average 55,940) are not significantly greater than the numbers shown in table 3 for similar controls given only one stimulating infection (average 52,259). It seems, therefore, that there was total immunity to muscle invasion of larvae produced by the last two stimulating infections.

EXPERIMENT 3.—*The effect of alcohol on immunity acquired from four stimulating infections of 300 larvae each.*—Experimental conditions were similar to those of the preceding experiment until the time of the third stimulating infection. Seven days after this infection alcohol treatment was renewed and continued for

seven days in the re-alcoholic group. Twenty-four hours later all of the animals received the final stimulating infection of 300 larvae. Alcoholic treatment was discontinued at this point so that the schedule of alcohol dosage was identical with that of the previous experiment. The immunity developed by the various mice was challenged seven days later by an infection of 900 larvae. As a control on the number of adults developing from this infection, five male mice were included which had not received the stimulating infections. All animals were killed seven days after receiving the challenging infection. The results, except for the control group given only the challenging infection, are given in Table 5.

There is a striking difference in the numbers of adults in the re-alcoholics (average 542) and controls (average 76) given the same number of larvae at infections. Statistical analysis shows the ratio of the observed difference to its standard error is 7.88. An even more striking difference is noted in the numbers of adults recovered from these controls (average 76) and from the five males not given the four stimulating infections (average 744). These figures are highly significant as

TABLE 5.—*Showing the numbers of adults and larvae in alcoholic and control mice given four stimulating infections of 300 larvae each and killed one week after a challenging infection of 900 larvae*

No. mice	No. adults in intestine (average)	Average ratio to no. larvae in challenging infection (900)	No. larvae in muscles (average)	Average ratio to no. larvae in three stimulating infections (900)*
A. X-alcoholic mice				
5	230	0.26	101,155	112.4
B. Re-alcoholic mice				
5	542	0.60	107,050	118.9
C. Control mice given saline				
6	76	0.08	54,115	60.1

* Fourth stimulating infection not involved.

the ratio of the observed difference to its standard error is 21.7. Recovery of larvae was significantly greater in the x-alcoholics and re-alcoholics than in their controls, average ratios 112.4, 118.9, and 60.1. These ratios were obtained as described above for the previous experiment, i.e., by dividing the number recovered by the number given in three stimulating infections (900). The fourth stimulating infection was not involved since it was given less than 30 days before autopsy.

DISCUSSION

Alcohol as given above was shown to have no effect on natural resistance of mice to infection with *Trichinella spiralis*. By way of comparison, previous work on *Hymenolepis* had shown considerable impairment of natural resistance following similar alcoholic treatment (Larsh, 1945). The effects of the alcohol treatment were quite similar in both studies, including loss of appetite, failure to gain weight, loss of hair gloss, and recurrent diarrhea. Extension of the alcohol treatment prior to infection with *Trichinella* increased the severity of these effects but did not, as compared with controls, increase the percentage development of adults or larvae. It is of interest that the percentages for these mice compare favorably with those for untreated mice reported by Culbertson (1942).

The effect of the alcohol on acquired immunity to *T. spiralis* was shown to vary with the number of stimulating infections. After one stimulating infection, no effect of the drug could be demonstrated. This was true despite the fact that one group of mice (re-alcoholics) was given alcohol both before and after the stimulating infection. Although the experimental conditions are not strictly comparable, it has been shown that alcohol given prior to infection with *Hymenolepis* definitely inhibited the immunity to reinfection (Larsh, 1946).

It is worth noting that the non-alcoholic mice that received one prior infection showed a 40.0 per cent development of adult worms from the challenging infection (table 3), whereas this development had been shown to be 65.4 per cent in non-alcoholic mice that had not received a previous infection (table 1). This may be an indication, therefore, that some immunity was developed as a result of the one stimulating infection. Even so, this degree of immunity would be slight as compared with that reported by Culbertson (1942). Using fewer larvae for infections, he demonstrated a striking immunity in mice as a result of a single infection (8.6 per cent development of adults in immunized mice and 55.8 per cent in controls).

After three stimulating infections, the alcohol had no effect on the immunity acquired to the intestinal phase of the parasite. At first glance the low percentage of adults found in all the mice would seem to indicate a rather striking degree of immunity (table 4). However, it must be noted that these animals were killed two weeks after the challenging infection and undoubtedly had lost large numbers of worms before autopsy. While there was no evidence that the alcohol interfered with the development of immunity to adults, there was evidence of drug interference with immunity to the larvae, since significantly greater numbers were found in the alcoholic mice.

After four stimulating infections, the alcohol was shown to have interfered with the development of immunity to both the adults and larvae. The experimental set-up was quite similar to that of the previous experiment except for the addition of the fourth stimulating infection and an increase in the size of the challenging infection (from 300 to 900 larvae). It is possible that this additional number of larvae resulted in a greater breakdown in the immunity of the alcoholic mice, thus accounting for the more striking demonstration of the drug's effect.

In the case of *Hymenolepis,* there is a good indication that alcoholic interference with immunity is related to the effect of the alcohol in reducing normal food intake, consequently producing hypovitaminosis. Although the present work sheds no light on the mechanism of this interference in the case of *T. spiralis,* there was noted the same reduction in food intake. This problem should be studied further because alcohol drains the vitamin A from its stores in the liver and other tissues (Clausen, *et al,* 1942), and because the lack of this vitamin is known to reduce the resistance of rats to *T. spiralis* (McCoy, 1934).

SUMMARY

Experiments were performed with mice to determine the effect of alcohol on natural and acquired immunity to *Trichinella spiralis.* Under the experimental conditions, it was not possible to demonstrate any effect of alcohol on natural resistance even in instances in which the alcoholic animals exhibited striking debilitation. The same was true regarding the effect on immunity acquired from one stimulating

infection In both cases, the numbers of adults and larvae were similar in alcoholic and control mice.

By using repeated stimulating infections, it was possible to show that the alcohol had a striking effect on the immune response. All alcoholic animals showed significantly greater numbers of adults and larvae than the controls, but the greatest parasite burden was shown by the mice given alcohol both before and after the stimulating infections.

References

Clausen, S. W., Baum, W. S., McCoord, A. B., Rydeen, J. O., and Breese, B. B. 1942 The mobilization by alcohols of vitamin A from its stores in the tissues. J. Nutrition. **24**: 1–14.

Culbertson, J. T. 1942 Active immunity in mice against *Trichinella spiralis*. J. Parasit. **28**: 197–202.

Gould, S. E. 1945 Trichinosis. Charles C. Thomas Co., Springfield, Illinois. 356 pp.

Larsh, J. E., Jr. 1945 Effects of alcohol on natural resistance to the dwarf tapeworm in mice. J. Parasit. **31**: 291–300.

———1946 The effect of alcohol on the development of acquired immunity to *Hymenolepis* in mice. J. Parasit. **32**: 72–78.

———1947 The role of reduced food intake in alcoholic debilitation of mice infected with *Hymenolepis*. J. Parasit. **33**: 339–344.

McCoy, O. R. 1934 The effect of vitamin A deficiency on the resistance of rats to infection with *Trichinella spiralis*. Amer. J. Hyg. **20**: 169–180.

Rappaport, I. 1943 A comparison of three strains of *Trichinella spiralis*. II. Longevity and sex ratio of adults in the intestine and rapidity of larval development in the musculature. Amer. J. Trop. Med. **23**: 351–362.

COURSE OF *TRYPANOSOMA LEWISI* INFECTIONS IN WHITE RATS TREATED WITH SODIUM SALICYLATE*

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Taliaferro and Taliaferro (1922) and Taliaferro (1923) have charted the course of *Trypanosoma lewisi* infection in rats. The presence of the parasites stimulates the production of two immune bodies: one is ablastin, the reproduction-inhibiting reaction product, and the other trypanolysin (*v.* Taliaferro, 1941). Failure of the reproduction-inhibiting antibody, manifested by prolongment of the reproductive phase, has been brought about experimentally by pantothenate deficiency in the host (Becker, Manresa and Johnson, 1943; Becker, Taylor, and Fuhrmeister, 1947) and by treating the host with sodium salicylate (Becker and Gallagher, 1947). The desirability of charting infections influenced with adequate but not excessive doses of salicylate and of studying the blood cell relationships in treated infections led to the present study.

MATERIALS AND METHODS

The host animal was the inbred Wistar A white rat. The strain of *T. lewisi* was that previously employed by Becker and his collaborators. The strain in the past had produced lighter infections than other strains, but the usual developmental stages appeared in the blood. Sodium salicylate (C. P.) was dissolved in distilled water in the concentration of 90 mg. per cc. Required amounts were given by means of a No. 8 soft rubber catheter, attached to a syringe, and introduced through the mouth directly into the rats' stomachs. The daily dose was approximately 45 mg. of sodium salicylate per 100 g. of rat weight.

The rats used in the study were divided into four groups. Group A rats were not infected with trypanosomes and did not receive sodium salicylate. Those in Group B were also parasite-free, but received sodium salicylate. Group C rats were infected with trypanosomes, but did not receive sodium salicylate treatment. The rats in Group D were injected with *Trypanosoma lewisi* and were administered sodium salicylate. On the zero day of the experiment the rats of C and D each received intraperitoneally 1 cc. of blood diluted with normal saline solution containing at least 100,000 trypanosomes. On zero day the rats of Groups B and D received their first treatment with salicylate.

Blood, for making smears and counts, was taken from the tips of the rats' tails, that for the erythrocyte and trypanosome counts being diluted with Hayem's solution, and that for the leucocyte count with Tuerk's solution. Erythrocyte and leucocyte counts were made by the standard haemacytometer methods. Trypanosomes were also counted by the red cell method except that at least twenty fields were counted for greater accuracy. No correction was made in the white cell counts for nucleated red cells.

Blood smears were stained in Wright's. Differential white cell counts and

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studies on trypanosomes were made on the stained smears. Two hundred leucocytes were counted differentially on each slide. The criterion of reproduction of trypanosomes was whether the blepharoplast was dividing or had divided. Usually 500–1000 trypanosomes were counted on each smear. Rarely, when the parasites present were too sparse to make this possible, fewer were counted.

TABLE 1.—*Trypanosome numbers, mean lengths (microns), standard deviations (microns), coefficients of variation and percents of dividing forms in untreated and treated infected rats.*

Rat Number	Kind of Data	Days after inoculation with *T. lewisi*									
		3	5	7	10	12	14	16	19	21	23
5* Treated	Thous. of Tryps. /mm³	0.0	0.0	2.5	112.5	360.0	270.0	162.5	120.0	32.5	0.0
	Mean length			24.0	29.6	28.6	30.6	29.2	31.2	30.4	31.1
	Std. Dev.			5.06	5.25	3.80	3.25	4.71	5.47	4.18	1.05
	C. V. %			21.1	17.7	13.3	10.6	16.1	17.5	10.5	3.4
	Div. Forms %			0.0	1.6	0.4	1.9	1.6	0.0	0.8	0.0
6* Treated	Thous. of Tryps. /mm³	0.0	0.0	5.0	77.5	325.0	175.0	155.0	180.0	55.0	5.0
	Mean length				26.2	27.9	29.5	27.6	28.7	31.2	29.5
	Std. Dev.				6.87	3.57	4.33	5.80	5.34	5.77	1.84
	C. V. %				25.8	12.8	14.7	21.0	18.6	18.5	6.2
	Div. Forms %				3.75	2.00	6.60	3.60	0.20	1.00	0.0
16† Treated	Thous. of Tryps. /mm³	7.5	55.0	180.0	305.0	13 227.0		167.5	110.0		0.0
	Mean length	30.2	27.7	27.1	30.9	27.9		30.2	28.3		
	Std. Dev.	6.31	6.14	6.16	5.26	6.12		5.51	5.37		
	C. V. %	20.9	22.2	22.7	17.0	21.9		14.9	19.0		
	Div. Forms %	1.0	0.6	0.2	0.8	1.3		0.6	1.0		
17† Treated	Thous. of Tryps. /mm³	2.5	80.0	167.5	275.0	322.5		242.5	90.0		2.5
	Mean length	27.6	28.5	29.8	31.0	29.3		30.4	27.5		
	Std. Dev.	6.14	7.89	6.40	5.01	6.02		5.57	5.67		
	C. V. %	22.2	27.7	21.1	16.2	20.5		18.3	20.6		
	Div. Forms %	1.0	1.5	0.4	0.5	0.7		1.2	0.8		

Rat Number	Kind of Data	Days after inoculation with *T. lewisi*							
		3	5	7	10	13	16	19	23
12 Untreated	Thous. of Tryps. /mm³	10.0	50.0	5.0	10.0	2.5	1.25	0.0	0.0
	Mean length	28.4	29.2	31.5	30.9	29.2	28.1		
	Std. Dev.	5.39	4.15	1.42	2.39	1.65	0.6		
	C. V. %	19.0	14.2	4.5	7.7	5.7	2.1		
	Div. Forms %	1.5	0.3	0.0	0.0	0.0	0.0		
21‡ Treated	Thous. of Tryps. /mm³	0.0	12.5	110.0	312.5	505.0	15 1205.0		
	Mean length		24.4	28.8	25.6	26.3	24.1		
	Std. Dev.		5.54	6.58	6.81	8.41	5.83		
	C. V. %		22.7	22.8	26.6	32.0	24.3		
	Div. Forms %		0.25	0.2	2.3	1.5	0.5		
23 Untreated	Thous. of Tryps. /mm³	2.5	35.0	60.0	27.5	40.0	22.5		
	Mean length	22.8	23.2	32.4	29.75	30.05	29.05		
	Std. Dev.	5.23	5.72	2.62	1.76	1.12	1.10		
	C. V. %	22.9	24.7	8.1	5.9	3.7	3.8		
	Div. Forms %	2.0	1.4	0.0	0.0	0.0	0.0		

* Last treatment of sodium salicylate given on 14th day.
† Last treatment of sodium salicylate given on 18th day.
‡ Last treatment of sodium salicylate given on 14th day. Rat died on 15th.

Taliaferro and Taliaferro (1922) used the coefficient of variation for lengths as the measure of the intensity of reproduction going on in the parasite population at a particular time. Becker and Lysenko (1948) used the standard deviation without degrees of freedom for the same purpose. Both standard deviations and coefficients of variation appear in Table 1 of the present report. Seventy trypanosomes were drawn from each slide with the camera lucida over a 12x eyepiece and oil im-

mersion lens except that in the case of Rat #12 on the 16th day of the infection the number was 35.

RESULTS

The administration of sodium salicylate had no effect on the leucocyte or erythrocyte counts of rats not infected with *T. lewisi*. Rats in Group C, infected with *T. lewisi* but receiving no salicylate, also exhibited normal leucocyte counts throughout the course of the experiment. The red blood cell count remained normal in this group except in Rat #12. In this rat anemia developed early in the infection, but the rat recovered before the trypanosome population reached its height, indicating a cause for the anemia other than the trypanosome infection.

Group D, infected with *T. lewisi* and treated with sodium salicylate, developed anemia of varying degrees of intensity. Only one rat (#21) succumbed. It died on the sixteenth day of the infection with a severe anemia and trypanosome population of over one million per mm^3. Other effects noted in the group were greater densities of trypanosome population and increased persistence of the parasites in the blood. Usually, however, the parasites showed a decline in density before the salicylate was discontinued.

It has long been known that eosinophilia often develops in cases of toxin production by various types of parasites. For this reason, differential leucocyte counts were made on all smears. There was in no case an indication of eosinophilia. There was, however, an indication that under treatment certain infected rats developed more basophils in the blood stream than would normally be present. The rats in Group A had an average of 0.245% basophils; Group B, 0.423%; Group C, 0.506%; and Group D, 0.682%. There seemed to be no other significant variation in the leucocytic formula.

Density, mean length, standard deviations, coefficients of variation and percents of actually dividing forms are recorded in Table 1. Standard deviations are shown in the graphs (Figs. 1–4). The same scales were used for all figures except Fig. 3. Here, the same scales were utilized for the standard deviation and mean lengths as in the other graphs, but the number scale was halved because of the tremendous population of parasites developed by Rat #21. The graph (Fig. 4) and Table 1 show that standard deviations for the untreated infected rats dropped rapidly during the first days of the experiment. In the treated rats, however, the deviations usually remained high for as long as the salicylate treatment was continued (Figs. 1, 2, 3). The standard deviations for the trypanosomes in Rat #21 showed a drop before the salicylate was stopped. It is impossible to say whether this would have continued, because the rat died. Counts and measurements were continued on the trypanosomes in Rats #5 and 6 some time after the cessation of the salicylate treatment. Within 4 or 5 days the standard deviation began to fall, indicating a sharp drop in the rate of reproduction.

Also shown in Table 1 and Figs. 1, 2, 3, and 4 is the fact, previously mentioned, that the numbers of trypanosomes reached a greater density in treated than in untreated rats. Later, even when the trypanosome population began to decrease, the density of the parasites was still comparatively high. The explanation for declining numbers is, of course, a more rapid destruction by trypanocidal forces than replacement by reproduction.

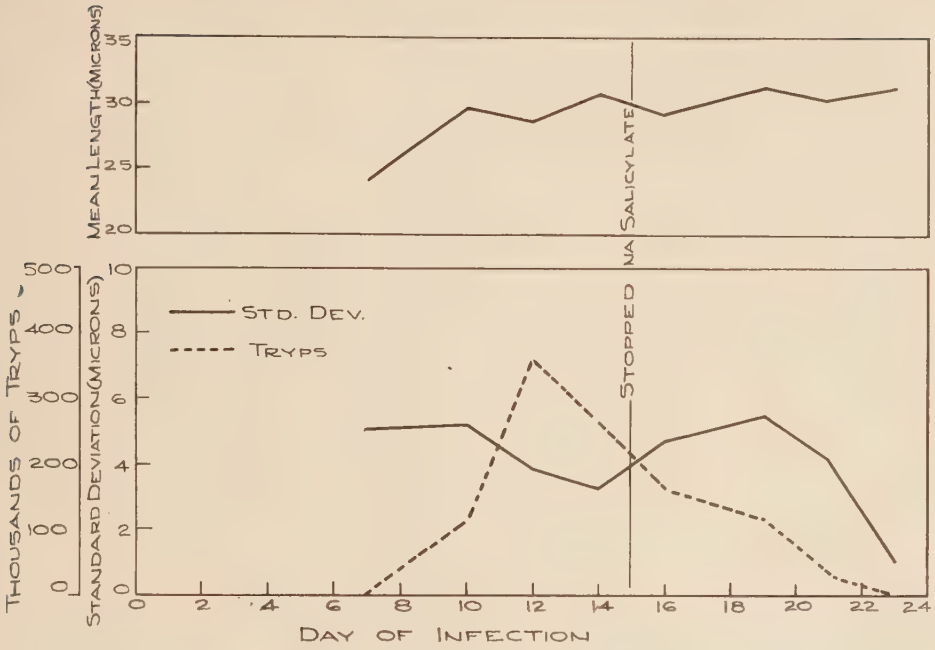

FIG. 1. Mean length of trypanosomes, standard deviation of lengths, and thousands of trypanosomes/mm³ of blood in Rat #5, treated with sodium salicylate.

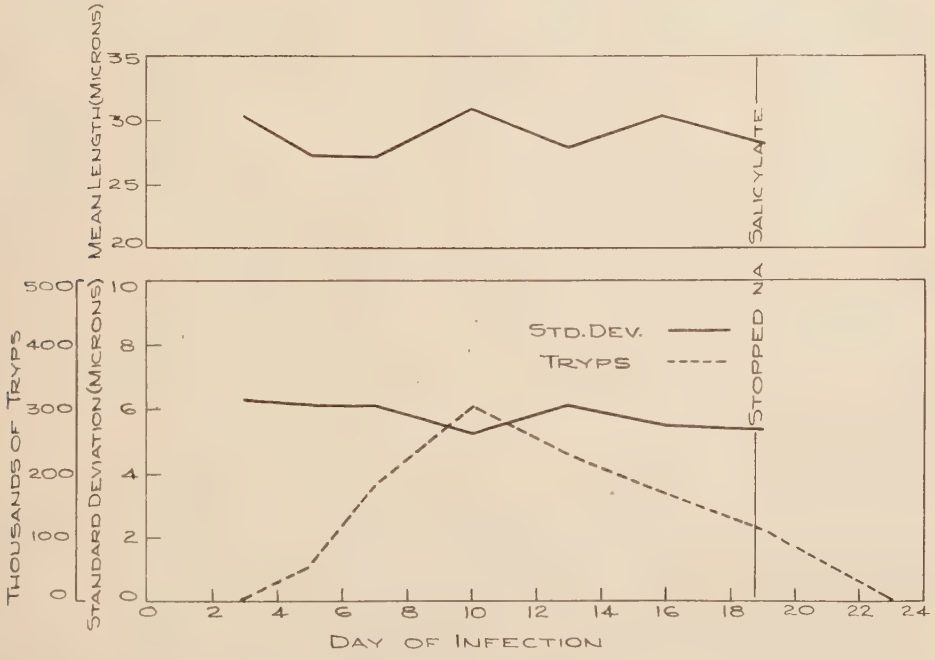

FIG. 2. Mean length of trypanosomes, standard deviation of lengths, and thousands of trypanosomes/mm³ of blood in Rat #16, treated with sodium salicylate.

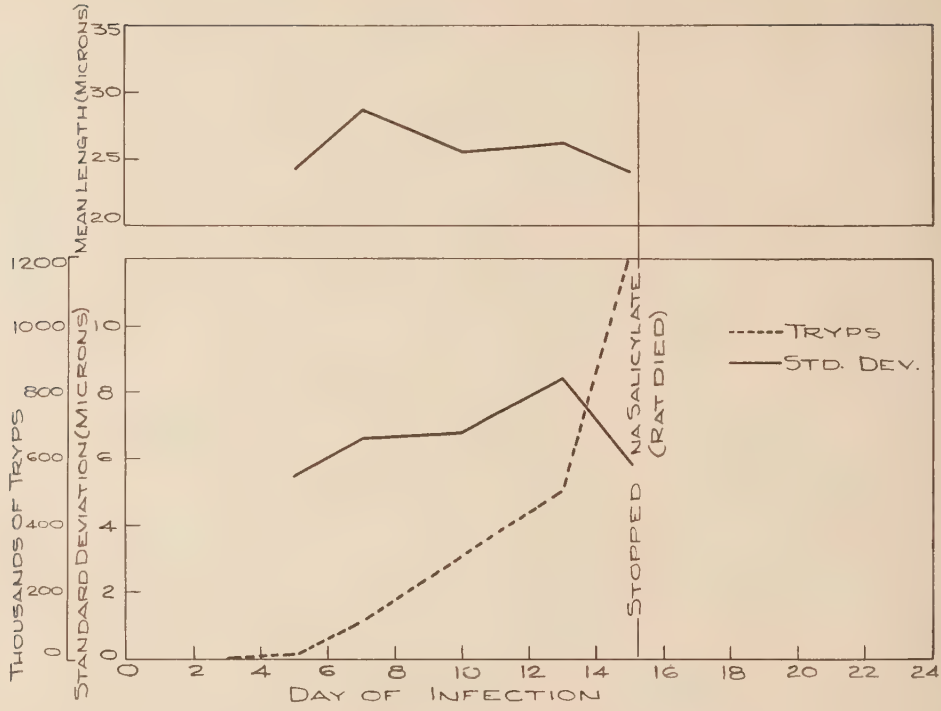

FIG. 3. Mean length of trypanosomes, standard deviation of lengths, and thousands of trypanosomes/mm^3 of blood in Rat #21, treated with sodium salicylate.

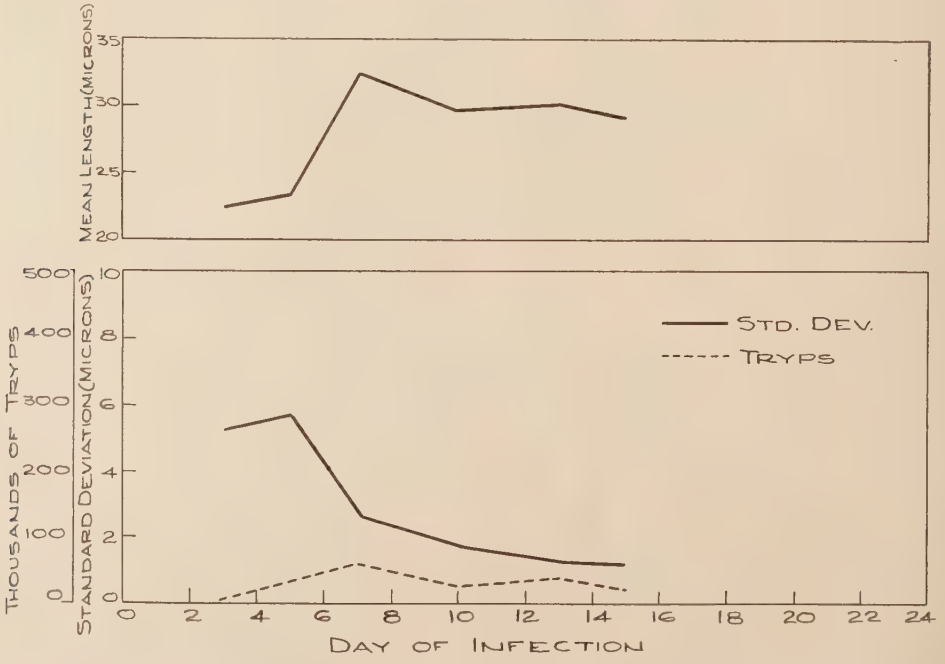

FIG. 4. Mean length of trypanosomes, standard deviation of lengths, and thousands of trypanosomes/mm^3 of blood in Rat #23, untreated.

The percent of division forms in the untreated rats rapidly dwindled to zero, sometimes as early as the fifth day of the infection. In the treated rats there was much variation in the percent of division forms recorded throughout the lengthened cycle. After the initial drop in the number of flagellates there was usually an increase, occasionally a sharp one, in the percent of division forms.

DISCUSSION

The most interesting data of the investigation were those showing that reproduction of the trypanosomes continued actively in the infected rats of Group D so long as sodium salicylate treatments were administered and for 4 or 5 days after their discontinuance. This was in contrast to the limited reproduction period of a few days duration in untreated, infected control rats. The antibody whose action was counteracted by sodium salicylate was the reproduction-inhibiting antibody, ablastin. That this was the one affected and not the trypanocidal antibody was shown by the fact that the amount and rate of reproduction, indicated by standard deviation, remained high so long as salicylate was administered. In contrast to this the parasite population became less dense regardless of the continuation of the treatment, indicating that the trypanocidal factor was active.

Other data, inconclusive in themselves, substantiated the conclusion that the reproduction-inhibiting factor was the antibody affected by the salicylate. There was admittedly much variation in the percentage of dividing forms of *T. lewisi* in Group D. There was usually, however, an increase in the percentage of dividing forms following the initial drop in the parasite population.

The data also demonstrated the fact that neither salicylate treatment nor *T. lewisi* infection alone significantly affected the density of erythrocytes in the blood of the rat. When infected rats were treated with sodium salicylate, however, they developed more or less severe anemia. The appearance of the anemia was associated with increased density of the parasite and prolongment of the reproductive phase, but the true cause of the anemia is not known. It is hoped that it will soon be possible for us to study the tissue reactions.

CONCLUSIONS

1. A dosage regimen of 45 mg. of sodium salicylate per 100 g. of rat host, administered daily commencing on the inoculation date, is about the optimum for studying the effect of the drug on the course of *Trypanosoma lewisi* infection.

2. Charting of such treated infections revealed (a) an extended period of parasite reproduction terminating 4 or 5 days after the discontinuance of salicylate administration and (b) heavier infections showing a steady decline in parasite density after the peak had been reached.

3. The prolongment of reproduction was due to interference with either the formation or action of ablastin, the anti-reproductive antibody of Taliaferro.

4. The decline in numbers despite continuing reproduction was due to the persistent action of the trypanocidal antibody in the presence of salicylate.

5. Neither the trypanosome infection nor the drug at the above dosage by themselves separately significantly affected the erythrocyte count.

6. The blood picture in infected treated rats was as follows: (a) heavy parasitemia, (b) actual division of trypanosomes long after they would normally have

ceased to reproduce, (c) red cell anemia, (d) light but definite basophilia and (e) no eosinophilia.

Literature Cited

Becker, E. R., and Gallagher, L. 1947 Prolongment of the reproductive phase of *Trypanosoma lewisi* by the administration of sodium salicylate. Ia. St. Coll. Jour. Sci. **21:** 351–362.

——— and Lysenko, M. G. 1948 Reinfection with *Trypanosoma lewisi* and recurrence of reproduction in recovered and near-recovered rats. Ia. St. Coll. Jour. Sci. **22:** 239–255.

———, Manresa, M., and Johnson, E. M. 1943 Reduction in the efficiency of ablastic action in *Trypanosoma lewisi* infection by withholding pantothenic acid from the host's diet. Ia. St. Coll. Jour. Sci. **17:** 431–441.

———, Taylor, J., and Fuhrmeister, C. 1947 The effect of pantothenate deficiency on *Trypanosoma lewisi* infection in the rat. Ia. St. Coll. Jour. Sci. **21:** 237–243.

Taliaferro, W. H. 1923 A study of size and variability throughout the course of pure line infections with *Trypanosoma lewisi.* J. Exp. Zool. **37:** 127–167.

———1941 The immunology of the parasitic protozoa. Chapt. XVIII in Calkins, G. N., and Summers, F. M. *Protozoa in Biological Research.* Columbia University Press. New York.

———, and Taliaferro, L. G. 1922 The resistance of different hosts to experimental trypanosome infections, with special reference to a new method of measuring resistance. Amer. J. Hyg. **2:** 264–317.

A NEW SPECIES OF *EURYTREMA* (TREMATODA: DICROCOELIIDAE) FROM THE SLATE-COLORED JUNCO

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In the course of routine examinations for parasites of birds and other animals collected in the vicinities of Lafayette and Greencastle, Indiana, it was found that the Slate-colored Junco, *Junco hiemalis hiemalis* (L.) occasionally harbored one or more trematodes in its gall bladder. This *Junco* generally spends only the winter months in Indiana and then migrates into Canada for the warmer portion of the year. Test and Test (1931, 1934) state that these birds usually arrive in Tippecanoe County near the middle of October, sometimes earlier, and depart in late April to early May. It is likely that the Juncos obtain their infections in Canada, although there is no definite information upon this point. The fact that immature forms have never been found in our experience, lends some support to this hypothesis. In all, sixteen specimens of *J. hiemalis hiemalis* (L.) were examined over a period of twelve years and four were positive for this particular trematode infection. The number of parasites per bird varied from one to five, with two birds having five each, one having three, and one having one. When five worms were present, they nearly filled the gall bladder of this small bird.

In working out the anatomy of the parasite, living material, whole mounts, and serial cross, sagittal, and frontal sections were used. The whole mounts were stained with paracarmine and the serial sections were stained with eosin and Delafield's hematoxylin. The anatomical characteristics of this trematode indicate that it is a new species to which the name *Eurytrema alveyi* is given.

Eurytrema alveyi n. sp.

The living worms are light pink in color, the suckers being somewhat more intensely pink than the rest of the body. The intestinal ceca appear bright yellow from the bird's bile which they contain. The cuticula bears small protuberances in the anterior body region but is otherwise unarmed. The body is flattened and spindle shaped with a maximum width at the level of the gonads. The body length of five specimens varies from 2.61 to 4.67 mm, averaging 3.51 mm; the body width ranges from 1.13 to 1.84 mm, averaging 1.50 mm. The subterminal or nearly terminal oral sucker is approximately circular in outline and measures, on the average, 0.345 mm in diameter. The ventral sucker is usually wider than it is long and ranges from 0.32 to 0.49 mm in length by 0.43 to 0.54 mm in width. The pharynx is greater in length than in width and ranges from 0.13 to 0.24 mm in length by 0.06 to 0.15 mm in width. The esophagus is usually as long as or a little longer than the pharynx. The intestine bifurcates midway between the suckers, and the ceca extend posteriorly

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a considerable distance but, in most cases, do not reach the posterior end of the body. In one specimen, the intestinal ceca closely approach the posterior end of the body In sections, the walls of the intestines are irregular with small pouches extending laterally.

The two testes are lateral to and extend a short distance posterior to the ventral sucker. Their outline varies from smoothly oval to slightly lobed. The right testis varies in length from 0.21 to 0.32 mm; in width from 0.14 to 0.22 mm. The left testis varies in length from 0.19 to 0.26 mm; in width from 0.15 to 0.31 mm. The two vasa efferentia course anteriad and mediad and unite to form a vas deferens just before entering the cirrus sac. The cirrus sac contains a coiled, tubular seminal vesicle (Fig. 3) and a weakly developed cirrus. The "prostate" cells are few in number. In none of our specimens does the cirrus sac extend to the ventral sucker. Its anterior end terminates at the genital pore which is located in the mid-ventral region at the level of the pharynx. In most cases, the genital pore is ventral to the mid-region of the pharynx.

The vitelline glands are arranged in clusters which, for the most part, are lateral to the intestinal ceca. These glands extend from the level of the body immediately in front of the testes, or from the testes' level, posteriorly to within approximately one-fourth the length of the worm from the posterior end of the body. Two vitelline ducts course mediad at about the mid-body level and empty into a small vitelline reservoir which connects to the oviduct just before the latter enters Mehlis' gland (Figs. 1, 4).

The ovary usually is distinctly lobed but in one specimen it has a smooth outline. It varies in size from 0.23 to 0.39 mm in the anterior-posterior axis by 0.29 to 0.41 mm in the lateral axis. In all cases, it is larger than the testes. Usually it is located slightly to the left of the median body region, but in one specimen it is slightly to the right. The ovary, seminal receptacle and Mehlis' gland are located close together, usually partly overlapping. The oviduct, soon after leaving the ovary, is joined by the duct of the seminal receptacle (Fig. 4). The seminal receptacle is approximately one-third the size of the ovary. In living specimens viewed from the ventral side, sperm were seen moving in a clock-wise direction in the seminal receptacle. A short Laurers' canal, opening externally at the dorsal body surface, connects to the oviduct near the junction of the latter with the duct from the seminal receptacle. Just before entering Mehlis' gland, the oviduct receives the duct from the vitelline reservoir. After leaving Mehlis' gland, the slings of the uterus fill most of the body posterior to the ventral sucker. The most recently formed eggs are colorless or very light yellow, while the older eggs are dark brown. The eggs reach the outside through the genital pore whose location has been described. The eggs are oval in shape, operculated, without spines, and the older eggs contain a non-eyespotted miracidium. Measurements of twelve eggs from the gall bladder of the host were: length, 0.029–0.032 mm (av. 0.031); width, 0.0187–0.022 mm (av. 0.02) mm. They were similar in size and shape to older eggs still within the worm.

In living specimens, the entire excretory system (Fig. 6) was traced without difficulty because of the large size of all the tubules. The long, tubular excretory bladder is dorsally located in the body. It extends anteriad approximately one-third the length of the body. The two main collecting ducts connect to the excretory bladder near its anterior end and extend anteriorly and laterally to the level of the

gonads, where each subdivides to form anterior and posterior secondary tubes. Each secondary tube receives four capillaries which terminate in flame cells. These capillaries are about 0.0015 mm in width when not constricted. The formula expressing the excretory pattern is $2[(1+1+1+1)+(1+1+1+1)]$.

DISCUSSION

Several authors, Stunkard (1947), Denton (1944), McIntosh (1939), have expressed the opinion that the genera *Eurytrema* Looss and *Platynosomum* Looss are indistinguishable. For further discussion of this point and a fairly complete bibliography see Stunkard (1947). Both of these genera were described in 1907 by Looss but *Eurytrema* has priority and the validity of the genus *Platynosomum* remains to be determined by further research. A comparative study of the excretory bladder may be important in this regard. McIntosh (1939) stated that in *Eurytrema komareki* the excretory bladder is Y-shaped, but it is tubular in *E. alveyi* where, in living specimens, it was seen to contract as a tubular unit. Stunkard's (1947) description of the excretory vesicle of *E. vulpis* suggests that it is Y-shaped.

Denton (1944) fed the eggs of *Eurytrema procyonis* Denton, 1942, to the snail, *Mesodon thyroidus* (Say), and obtained mature mother sporocysts in 70 days. Later, daughter sporocysts containing cercariae ruptured from the snail host and were deposited upon vegetation and other objects. Stunkard (1947) cites unpublished data from Denton to the effect that direct infection of *Eurytrema* in birds does not occur. Maldonado (1945) states that *Platynosomum fastosum,* parasitizing cats in Puerto Rico, uses the lizard, *Anolis cristatellus,* as a second intermediate host. Lizards do not appear to be logical second intermediate hosts for species of *Eurytrema* parasitizing small, seed-eating birds.

Further determinations of life cycles in this genus will be of great value in clarifying the relationship of the various species.

E. alveyi resembles *E. ludovicianae* Petri, 1942, which parasitizes the liver of the rose-breasted grosbeak, *Zemelodia ludoviciana* (L.). However, *E. alveyi* has a more anteriorly located genital pore; the cirrus sac does not overlap the acetabulum; the vitellaria extend over much more of the body length; and the eggs are considerably smaller than in *E. ludovicianae.*

SUMMARY

A new species of *Eurytrema, E. alveyi,* is described from the gall bladder of the bird, *Junco hiemalis hiemalis* (L.) collected in Indiana, U.S.A.

Four of sixteen birds examined were found to harbor from one to five of the parasites.

The excretory bladder is tubular and the flame cell formula is $2[(1+1+1+1)+(1+1+1+1)]$.

REFERENCES

DENTON, J. F. 1944 Studies on the life history of *Eurytrema procyonis* Denton 1942. J. Parasit. **30:** 277–286.

MALDONADO, J. F. 1945 The life history and biology of *Platynosomum fastosum* Kossack, 1910 (Trematoda: Dicrocoeliidae) Puerto Rico J. Pub. Health Trop. Med. **21:** 17–39.

MCINTOSH, A. 1939 A new dicrocoeliid trematode, *Eurytrema komareki* n. sp. from the white footed mouse. Proc. Helm. Soc. Wash. **6:** 18–19.

PETRI, L. H. 1942 Two new dicrocoeliid trematodes from birds. Trans. Amer. Micr. Soc. **61:** 57–61.

STUNKARD, H. W. 1947 A dicrocoeliid trematode, *Euretrema vulpis* n. sp. *provis* from the pancreatic ducts of the red fox. J. Parasit. 33: 459–466.
TEST, L. A. and TEST, F. H. 1931 Local movements of birds. Proc. Ind. Acad. Sci. 40: 369–370.
TEST, L. A. and TEST, F. H. 1934 Birds of Tippecanoe County—IV. Proc. Ind. Acad. Sci. 43: 228–235.

EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida except where otherwise stated.

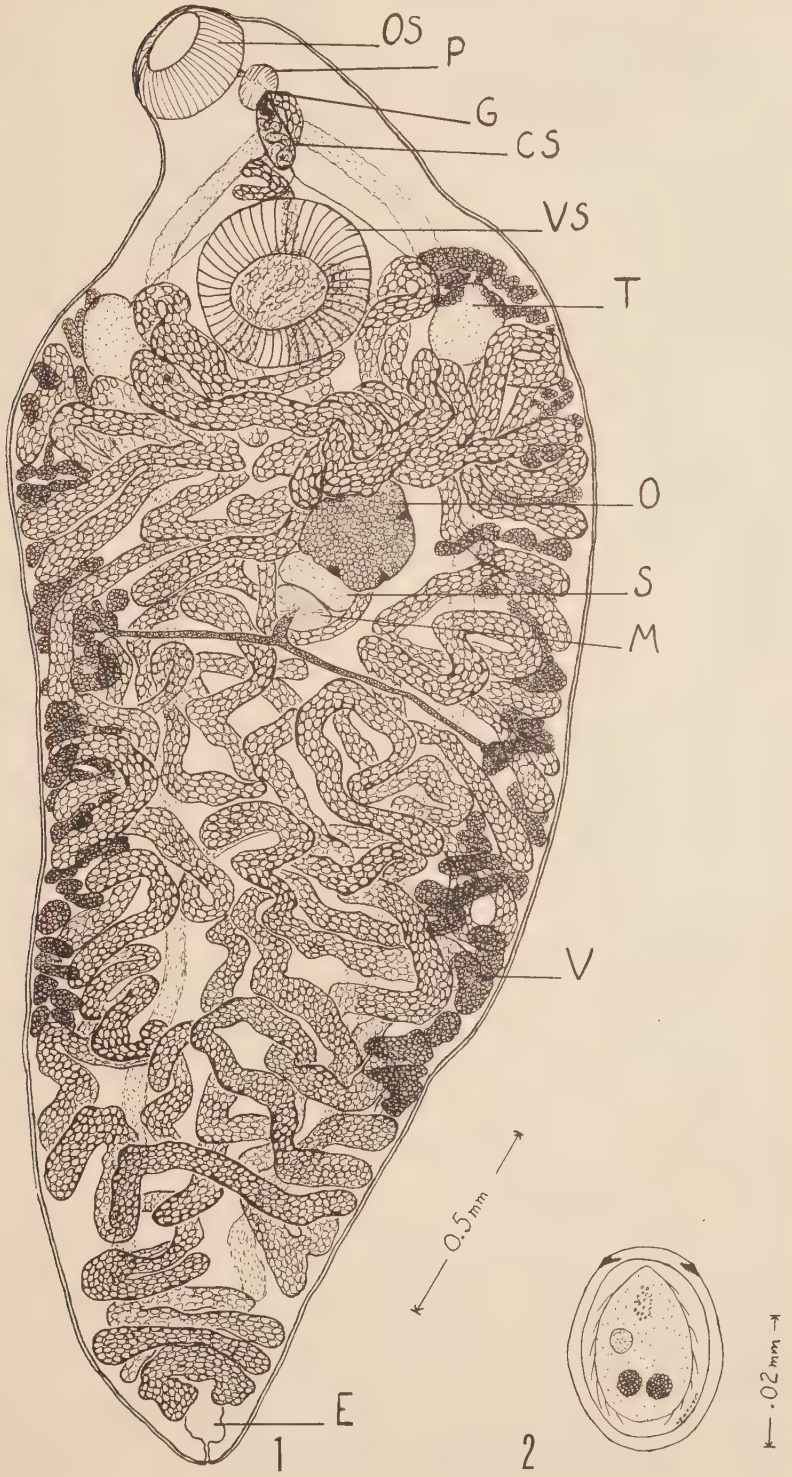
C cirrus
CD main collecting duct
CS cirrus sac
E excretory vesicle
F flame cell
G genital pore
L Laurers' canal
M Mehlis' gland
O ovary
OS oral sucker
OV oviduct
P pharynx
S seminal receptacle
SV seminal vesicle
T testis
V vitellaria
VR vitelline reservoir
VS ventral sucker

PLATE I

FIG. 1. *Eurytrema alveyi*—ventral view of entire specimen.
FIG. 2. Embryonated egg.

PLATE II

FIG. 3. Longitudinal section of cirrus sac showing sections of the coiled seminal vesicle and a portion of the cirrus.
FIG. 4. Diagrammatic representation of part of the female reproductive system.
FIG. 5. Anterior end of the excretory vesicle showing the connection of one of the main collecting ducts.
FIG. 6. A diagram of the excretory system.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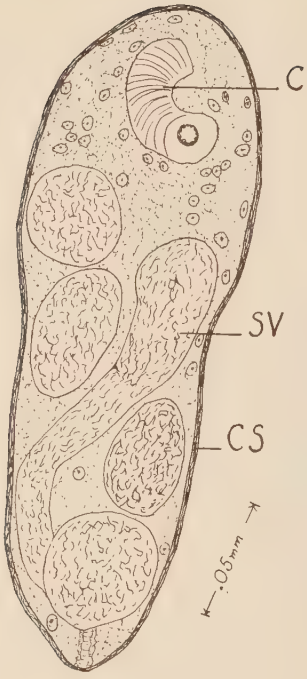

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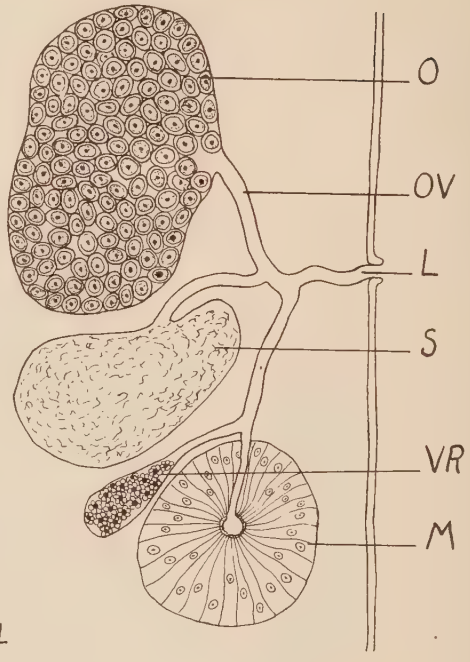

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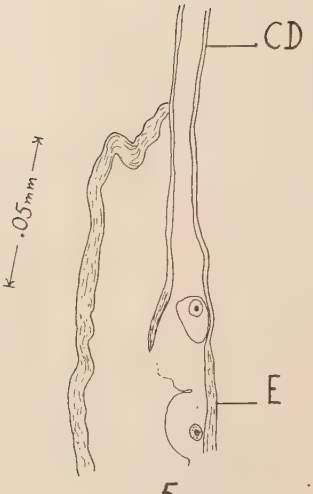

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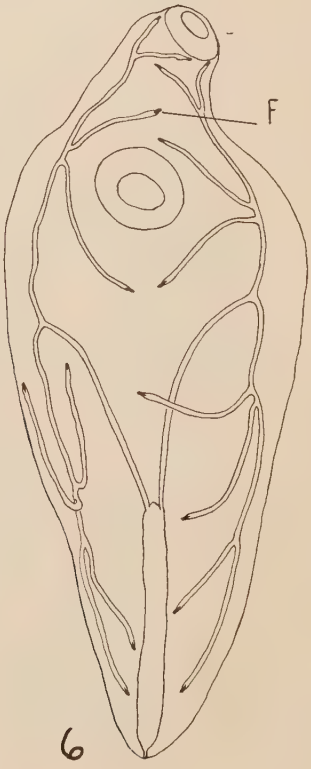

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TWO NEWLY DESCRIBED SPECIES OF MICROSPORIDIA FROM THE POTATO TUBERWORM, *GNORIMOSCHEMA OPERCULELLA* (ZELLER) (LEPIDOPTERA, GELECHIIDAE)[1]

EDWARD A. STEINHAUS AND KENNETH M. HUGHES

University of California, Berkeley

In 1945, Allen and Brunson reported the presence of a microsporidian in the hymenopterous parasite *Macrocentrus ancylivorus* Rohwer being propagated on the potato tuberworm *Gnorimoschema operculella* (Zeller). Later (1947), they designated the organism as a *Nosema* and pointed out that it infected not only the hymenopterous parasite but the host tuberworm as well.

What may be the same species of microsporidian was observed in 1946 in both species of insects as they were being reared in a University of California insectary being operated by the Division of Biological Control at Albany, California. According to Finney, Flanders, and Smith (1947), the infection in the insectary caused the emergence of tuberworm larvae to drop to nearly 50 per cent and the *Macrocentrus* emergence to drop to between 65 and 70 per cent. Approximately 45 per cent of tuberworm larvae still within the potatoes contained microsporidian spores. The disease was controlled by a modification of the method devised by Allen and Brunson (1947) in which the *Gnorimoschema* eggs are treated in a hot-water bath at 47° C. for 20 minutes.

Early in 1947 the Laboratory of Insect Pathology of the Division of Biological Control at the University of California began a detailed study of the microsporidian concerned. It was hoped that such a study would yield more data relative to the disease as it occurs in the insectary as well as furnish information as to the possibility of using the protozoan against certain destructive insects. Of first importance was the determination of the identity of the organism as well as the principal phases of its life cycle. Shortly after this work was underway it became evident that in addition to the *Nosema* species, another species of Microsporidia (a *Plistophora*) was also present in the tuberworm larvae being propagated in the insectary. This complicated the problem somewhat and it was decided to make a study of the life history of the second species along with that of the first. The purpose of the present paper is to report the results of these initial studies on the life histories of the two species.

Nosema destructor n. sp.

LIFE CYCLE

After the ingested spore germinates in the alimentary tract of the new host, the first stage to be recognized is that which corresponds to the amoebula stage of other descriptions. It is a body with an irregularly ovoid outline. There are two nuclei which sometimes do not show up very distinctly. The cytoplasm usually appears dense and stains rather heavily. A typical specimen is represented in fig. 1*A*. It is usually about 2.5 to 3.0 by 3.0 to 4.0 microns in size. The appearance of some

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[1] Contribution from the Laboratory of Insect Pathology, Division of Biological Control, College of Agriculture, University of California, Berkeley.

individuals in stained preparations (Fig. 1*B*) indicates that the organism is capable of amoeboid movement. Since all gradations in size, from the amoebulae just described to the largest of the intracellular schizonts, are found, it would appear that the transition from the amoebula to the intracellular form is one of simple growth with little noticeable alteration in form (Fig. 1, *A* to *F*). The shape of the latter stage is often more regular than that of the amoebula. The typical schizont (Fig. 1*F*) is a somewhat-rounded body with two prominent nuclei. When stained with Giemsa solution, the nuclei are bright red while the cytoplasm is blue.

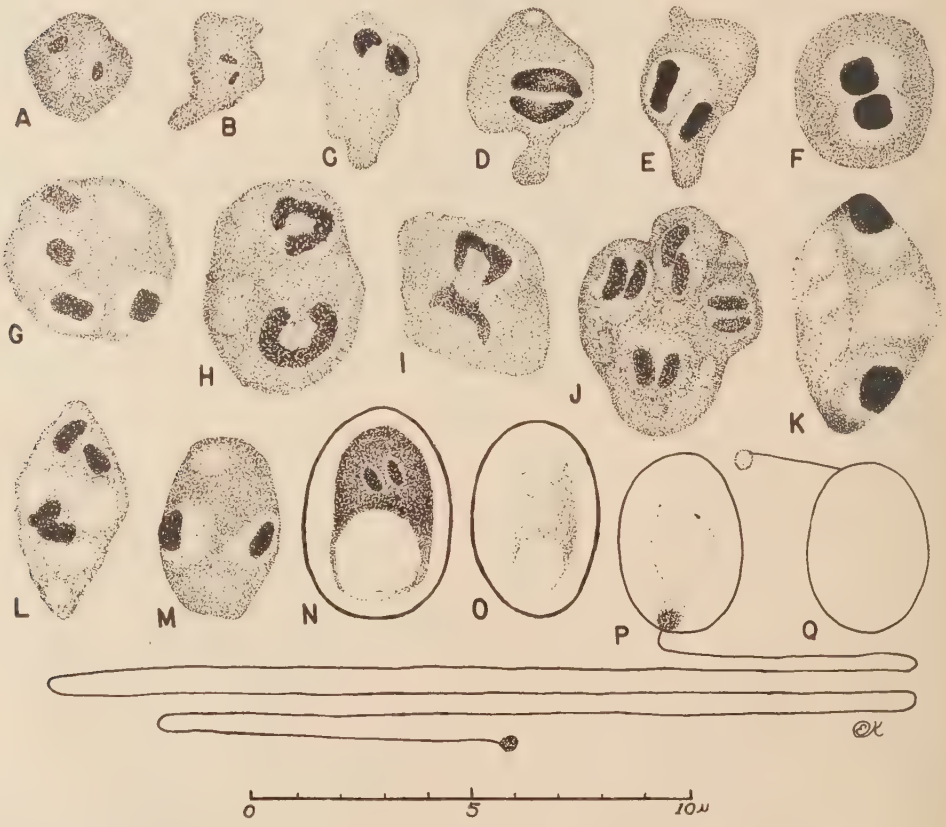


FIG. 1. Stages in the life cycle of *Nosema destructor* n. sp. *A–B*, amoebulae; *C–J*, schizonts; *K–L*, sporonts; *M*, probable sporoblast; *N*, young spore; *O*, mature spore; *P*, spore with fully extruded polar filament; *Q*, spore with partially extruded filament. (*A–O*, Giemsa stain; *P*, fresh preparation; *Q*, protargol stain.)

Two forms of schizonts may be recognized. In the one seen most commonly, the nuclei appear very dense and compact (Fig. 1*F*). In the other form, the nuclei appear more diffuse, often having an irregular ring shape (Fig. 1*H, I*). The true significance of this difference in nuclear appearance is not clear. Frequently, the larger schizonts are the ones with diffuse nuclei while the small or medium sized ones have compact nuclei. It is possible that the two types of nuclei represent the products of two different schizogonic divisions, although this is purely conjecture. Dividing forms with each type of nuclei have been seen. Schizonts having four

nuclei are frequently seen (Fig. 1*G*; Plate 1*D*). It is assumed that these are ones which are about to divide into two binucleate schizonts. The schizonts range in size from that of the amoebulae to as much as 9 microns in diameter in the case of 4-nucleate forms. In larger forms particularly, there is often a lightly staining region of cytoplasm surrounding the nuclei.

Another type of schizont, seen only occasionally, appears to indicate a different mode of division. These individuals are quite irregular in shape and have varying numbers of nuclei in them (Fig. 1*J*). The nuclei are frequently small and not too distinct. They usually appear to be grouped in pairs, although this grouping is not always apparent. The cytoplasm sometimes stains with a varying intensity in different regions, producing a mottled effect. Some of these have been seen to have breaks in the cytoplasm as if the entire body was starting to fragment into a number of individuals. It appears that these schizonts represent a mode of division in which the nuclei divide repeatedly without cytoplasmic division, followed by fragmentation of the cytoplasm to produce a number of new individuals simultaneously.

Typical sporonts are elongated, binucleate bodies with vacuolated, lightly staining cytoplasm (Fig. 1*K*). The nuclei are rather indefinitely shaped and often are not very distinct. The sporonts are usually about 5 or 6 microns in the longest dimension. Some are found to have 4 nuclei (Fig. 1*L*). The presence of those specimens with four nuclei would seem to indicate that a division may take place within the sporont stage. These tetranucleated forms are only rarely seen, however, and it is believed that in the majority of cases no such division takes place. Sporonts are not common in smear preparations. Apparently the sporont stage is of rather short duration, and, since in any one infected insect all stages of the parasite can usually be found at any one time, it is not often that a slide will contain many sporonts. In fact, it is not unusual for a slide to contain many schizonts and many spores, while no sporonts of the type described above are to be found. This has led to the hypothesis that the organism may go through a sporont stage in which it does not assume the elongated, vacuolated appearance, and hence in which it is indistinguishable from the schizont stage.

The sporoblast has not been demonstrated very definitely. Some structures similar to fig. 1*M* may be sporoblasts. They are similar in size and shape to a spore, and look similar to a young spore except that no spore wall is distinguishable. In order for a sporont to become a spore, it must undergo considerable condensation of the cytoplasm—quite possibly with the discarding of unwanted cytoplasm. In the young spore there is a relatively thin spore wall, the cytoplasm stains quite deeply and the nuclei are sometimes still visible (Fig. 1*N*). In the fully mature spore, the wall is quite thick, the cytoplasm stains only lightly and the nuclei are not visible with Giemsa stain (Fig. 1*O*).

The average dimensions of the spore are approximately 2.8 by 4.0 microns. One group measured gave dimensions from 2.25 to 3.00 microns wide by 3.00 to 4.75 microns long. Occasionally some spores are seen which appear to be about double the length of the normal spore but with no increase in width. Thus, one was measured at 2.81 by 6.00 microns. With silver impregnation methods (protargol), two nuclei can be seen in the mature spore. The same method has been used to demonstrate polar filaments after having applied pressure to the spore. The polar filaments thus demonstrated have a length of about 70 microns (Fig. 1*P*), and frequently

appear to have a small body attached to the distal end. What were believed to be incompletely extruded polar filaments have been seen occasionally in wet mounts. These usually appear as a short filament, only a few microns long, with a small ball on the distal end (Fig. 1*Q*). The same appearance has been noted in *Nosema ctenocephali* by Korke (1916) and in *Nosema* sp. by Ishiwata (1917). In these cases of partial extrusion of the polar filament, the filament seems to arise from a point to one side of the end of the spore (Fig. 1*Q*).

Since each sporont of the microsporidian with which we are concerned characteristically forms a single sporoblast which in turn forms a single spore, there is no question but that it belongs to the genus *Nosema* Naegeli. Occasionally, it appears that two sporoblasts are formed from each sporont, but such exceptions have been noticed in other species of *Nosema* (e.g., *Nosema carpocapsae* Paillot, 1939). A thorough comparison of the sporozoan with the descriptions of all other reported species of *Nosema* leaves little doubt but that we are dealing with a new species. For this reason the name *Nosema destructor* n. sp. is proposed for this microsporidian.

PHYSIOLOGICAL DATA

Potato-tuberworm larvae infected with *Nosema destructor* do not exhibit any particularly distinctive symptoms. The affected insects have a diminished appetite, are somewhat sluggish in movement, and may die prior to pupation. It is common, however, for infected larvae to pupate and to emerge as infected adults. Infected larvae may become stunted and somewhat more opaque and whitish in appearance as the result of an accumulation of spores in the body tissues. When a larva is heavily infected, the parasite may be found in almost any tissue of the insect's body. The primary seat of the infection, however, appears to be the fat body, the Malpighian tubes, and the silk glands.

Spores of *Nosema destructor* suspended in water and held in the refrigerator (4° C.) for 184 days produced an infection in 50 per cent of the test larvae. Since earlier experiments with the same suspension after 87 days produced infection in approximately 100 per cent of the larvae, decrease in the viability of the spores since the first test is indicated. Spores held on glass slides in the form of dried smears produced infection after 38 but not after 168 days.

Preliminary experiments on the blood picture of potato tuberworm larvae infected with the microsporidian compared with that of uninfected larvae, indicated that the infection causes some alteration in the differential counts of leucocytes and lymphocytes. The blood cells of healthy tuberworms appear to consist of approximately 67 per cent leucocytes and 33 per cent lymphocytes. Larvae infected with *Nosema destructor* have averages of about 40 per cent leucocytes and 60 per cent lymphocytes.

Whether or not the potato tuberworm, *Gnorimoschema operculella* (Zeller), is the principal natural host of *Nosema destructor* is not known. Insectary specimens of this insect are the only ones from which the microsporidian has been recovered. According to McCoy (1947), the presence of the protozoan in the hymenopterous parasite *Macrocentrus ancylivorous* Roh., is a fortuitous one. Experimentally, the larvae of a number of other insects are known to be susceptible to the oral administration of *Nosema destructor*. These include: *Colias philodice eurytheme* Bdvl., *Phryganidia californica* Pack., *Danaus plexippus* Linn., *Laphygma exigua* (Hbn.), *Perisierola emigrata* Roh., *Cremastus flavo-orbitalis* Cameron, *Pieris rapae* (Linn.)

and *Carpocapsa pomonella* (Linn.). In single experiments of several individuals each, larvae of *Tenebrio molitor* Linn. and *Estigmene acraea* (Dru.) failed to become infected after being fed suspensions of the microsporidian.

Plistophora californica n. sp.

LIFE CYCLE

The schizogonic stages of the *Plistophora* observed in the potato tuberworm larvae have not been definitely identified. In one area of one slide some bodies were

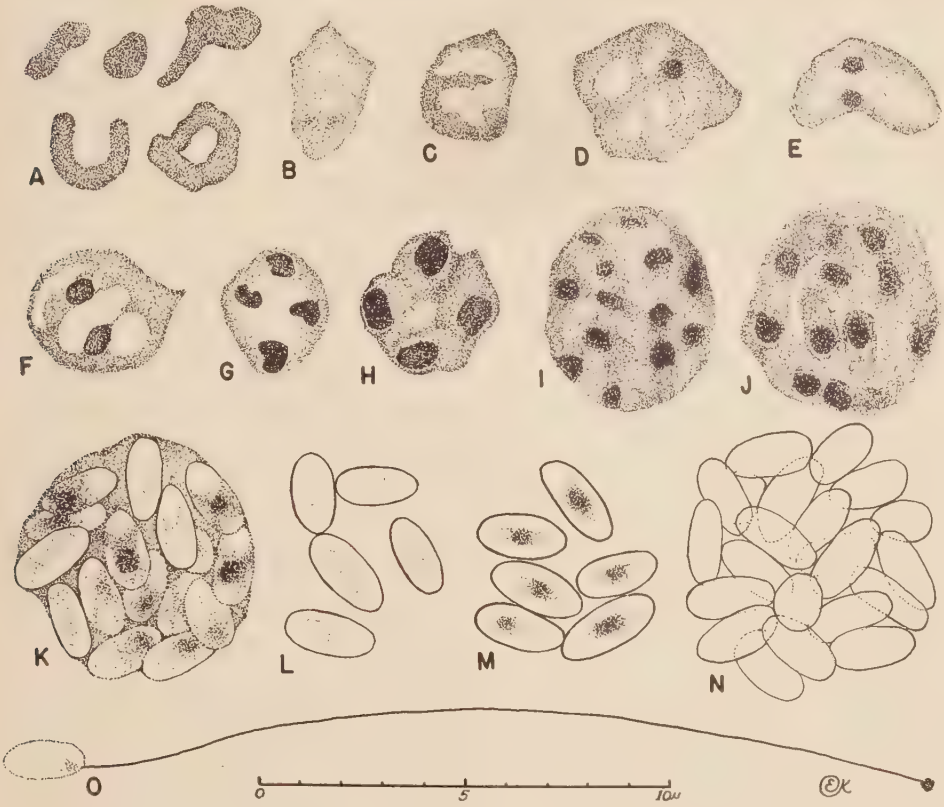


FIG. 2. Stages in the life cycle of *Plistophora californica* n. sp. *A*, probable schizonts; *B–G*, early sporonts; *H–K*, pansporoblasts; *L–M*, mature spores; *N*, packet of spores as seen in fresh preparation; *O*, spore with extruded polar filament. (*A–L*, Giemsa stain; *M* and *O*, protargol stain.)

seen which may represent the schizonts. These bodies, found in silk gland cells, are very irregular in shape. They were stained a nearly uniform blue with no nuclei or other internal structures visible. Some of the larger ones seemed to have a lighter area in the center. They range from 1 to 5 microns long and were seldom less than 1 micron wide. Examples are shown in fig. 2*A*. Since they have been found in only one instance, it is impossible to say whether or not they are a stage in the life cycle of the protozoan under consideration.

The first definitely recognizable stage of this organism is a rounded body fre-

quently with vacuolated cytoplasm and a variable number of nuclei visible. Examples of this stage with no nuclei visible are shown in fig. *2B* and *C*. If the apparently non-nucleated bodies described in the preceding paragraph as being possible schizonts are actually such, then the present structures probably represent an early sporont in which there is not yet a definitely formed or stainable nucleus. A sporont with a single nucleus is shown in fig. *2D* and a binucleate one in fig. *2E* and *F*. A sporont with four nuclei is shown in fig. *2G*. These sporonts range from 3 to 5 microns in diameter. In most cases, sporonts undergo further nuclear divisions until the ultimate pansporoblast is a many-nucleated structure. In at least some cases, however, as few as four spores may be formed. A four-sporoblast pansporoblast is shown in fig. *2H*. This structure appeared to have spore walls forming around the nuclei. It is unusual to find spores in groups composed of fewer than eight spores. In many cases, it appeared that more than 100 spores may result from one pansporoblast. The typical pansporoblast is a plasmodium-like structure containing many nuclei. The cytoplasm does not stain uniformly but appears to have light and dense areas. Usually these pansporoblasts vary between 6 and 10 microns in diameter (Fig. *2I, J*). It appears that each nucleus of the pansporoblast goes into the formation of a separate spore (Fig. *2K*). This is confirmed apparently by the observation that, in silver impregnation preparations of these spores, a single nucleus is seen.

The spores are fairly uniform in shape. They vary in length between 1.5 and 3.0 microns. The width ranges from 0.8 to 1.2 microns. The average dimensions are about 1.0 by 2.0 microns (Fig. *2L*). The polar filament, as seen in protargol preparations, averages about 20 microns in length. One polar filament measuring 27 microns has been seen with the electron microscope. What appears to be a minute ball or body may frequently be seen at the distal end of the extruded filament. It is possible that this structure may be nothing more than a loop in the end of the filament.

In fresh preparations, the spores are often found occurring singly. In some preparations, however, they are often seen still grouped in packets (Fig. *2N*). Whether or not these bundles are enclosed by a membrane is a point that the writers have not been able to determine. The spores composing such a group are held together for some time but, in a water preparation, they eventually are seen to break apart freeing the individual spores. With larger groups, it has been impossible to determine the exact number of spores contained. Those which appear to have about eight spores are seen frequently. Those which have about 16 are, perhaps, the most common. It is not unusual to find large packets which are estimated to contain more than 100 spores.

The fact that the microsporidian forms a variable number of sporoblasts (often more than 16) each of which becomes a spore, makes it clear that we are dealing with a species of the genus *Plistophora* Gurley. There is, in the literature, a slight variance of opinion as to the definition of this genus. As originally defined by Gurley (1893), the genus includes those species in which "the pansporoblast produces an inconstant but large number (always more than 8) of spores . . ." Kudo (1924), in his monograph, however, says of *Plistophora*, "Each sporont develops into many (more than 16) spores." In his textbook, "Protozoology," the same author modified the above statement to read "(often more than 16)." The dis-

crepancy between the above statements and the fact that our organism has been observed to form as few as four spores does not seem sufficiently great to justify the erection of a new genus to take care of so slight a difference in characteristics. A similar conclusion was apparently reached by Sprague and Ramsey (1942) when they named *Plistophora kudoi,* for they say, "Two to many spores develop in each sporont in the species under consideration. The former number is rare, for only one of this type was observed. Those containing six, eight, ten, or twelve spores are common, but the majority contain sixteen or more." It would appear that the genus *Plistophora* is in need of revision to more adequately embrace such species as *P. kudoi* Sprague & Ramsey and the one here described.

Since comparison of the characteristics of this species with the descriptions of other species of *Plistophora* failed to reveal any which were identical, it was decided to consider the present organism as a new species for which the name *Plistophora californica* n. sp. is proposed.

PHYSIOLOGICAL DATA

Larvae of the potato tuberworm lightly infected with *Plistophora californica* are devoid of any specific symptoms. Some sluggishness and slight loss of appetite may be exhibited by the affected insects. Heavily infected individuals may have a somewhat opaque and whitish appearance. Although almost any tissue may be infected the parasite occurs in greatest abundance in the fat body and the Malpighian tubes.

As has been demonstrated by Allen and Brunson (1947) with their *Nosema,* and by Finney, Flanders, and Smith (1947) with *Nosema destructor,* immersing the eggs from infected *Gnorimoschema* females in a hot-water bath at 47° C. for 20 minutes destroyed the microsporidian and permitted the larvae reared from them to be free of the protozoan. Similar results were obtained in our hands with *Nosema destructor,* but attempts to free the larvae of *Plistophora californica* by the same method were unsuccessful. Varying lengths of time up to 90 minutes were used in making the tests at 47° C. Slight if any reduction resulted in the number of infected larvae reared from the treated eggs.

As tested in the laboratory, the host range of *Plistophora californica* appears to be equally as broad as is that of *Nosema destructor.* The experimentally susceptible hosts include, in addition to *Gnorimoschema operculella* (Zeller), the following species: *Colias philodice eurytheme* Bdvl., *Phryganidia californica* Pack., *Danaus plexippus* Linn., *Macrocentrus ancylivorus* Roh., *Perisierola emigrata* Roh., *Cremastus flavo-orbitalis* Cameron, *Carpocapsa pomonella* (Linn.), *Vanessa carye* (Hüb.), *Copidosoma koehleri* Blanchard, and *Chrysopa californica* Coq. A single experiment in which larvae of *Tenebrio molitor* Linn. and *Estigmene acraea* (Dru.) were fed spore suspensions of the microsporidian, failed to show these two insects to be susceptible.

SUMMARY

Two new species of Microsporidia, causing infections in insectary-reared larvae of the potato tuberworm, *Gnorimoschema operculella* (Zeller), are described. The names *Nosema destructor* n. sp. and *Plistophora californica* n. sp. are proposed for them. In addition to the life histories of these two species, data relating to their host range, and certain physiological characteristics are given.

References

Allen, H. W., and Brunson, M. H. 1945 A microsporidian in *Macrocentrus ancylivorus*. J. Econ. Entomol. **38**: 393.

——— and ———1947 Control of *Nosema* disease of potato tuberworm, a host used in the mass production of *Macrocentrus ancylivorus*. Science **105**: 394.

Finney, G. L., Flanders, S. E., and Smith, H. S. 1947 Mass culture of *Macrocentrus ancylivorus* and its host, the potato tuber moth. Hilgardia **17**: 437–483.

Gurley, R. R. 1893 On the classification of Myxosporidia, a group of protozoan parasites infesting fishes. Bull. U. S. Fish Comm. **11**: 407–420.

Ishiwata, S. 1917 Note on a species of *Nosema* infecting *Attacus cynthia* Drury. J. Parasit. **3**: 136–137.

Korke, V. T. 1916 On a *Nosema* (*Nosema pulicis* n. sp.) parasitic in the dog flea (*Ctenocephalus felis*). Indian J. Med. Res. **3**: 725–730.

Kudo, R. R. 1924 A biologic and taxonomic study of the Microsporidia. Illinois Biol. Mono. **9**: 268 pp.

———1939 Protozoology. Charles C. Thomas, Baltimore. 689 pp.

McCoy, E. E. 1947 Elimination of a microsporidian parasite in the mass rearing of *Macrocentrus ancylivorus*. J. N. Y. Entomol. Soc. **55**: 51–55.

Paillot, A. 1939 Le carpocapse dans la region lyonnaise et les regions limitrophes. Ann. Epiphyties Phytogen. **5**: 199–211.

Sprague, V., and Ramsey, J. 1942 Further observations on *Plistophora kudoi*, a microsporidian of the cockroach. J. Parasit. **28**: 399–406.

Explanation of Plate

Photographs of *Nosema destructor* n. sp. and *Plistophora californica* n. sp. Giemsa stain.

A, Larvae of *Gnorimoschema operculella* (Zeller). Left, infected with *N. destructor* showing variation in size and color. Right, healthy larvae of the same age (×2/3).

B, Group of *N. destructor* schizonts (×2000).

C, Single schizont of *N. destructor* (×2000).

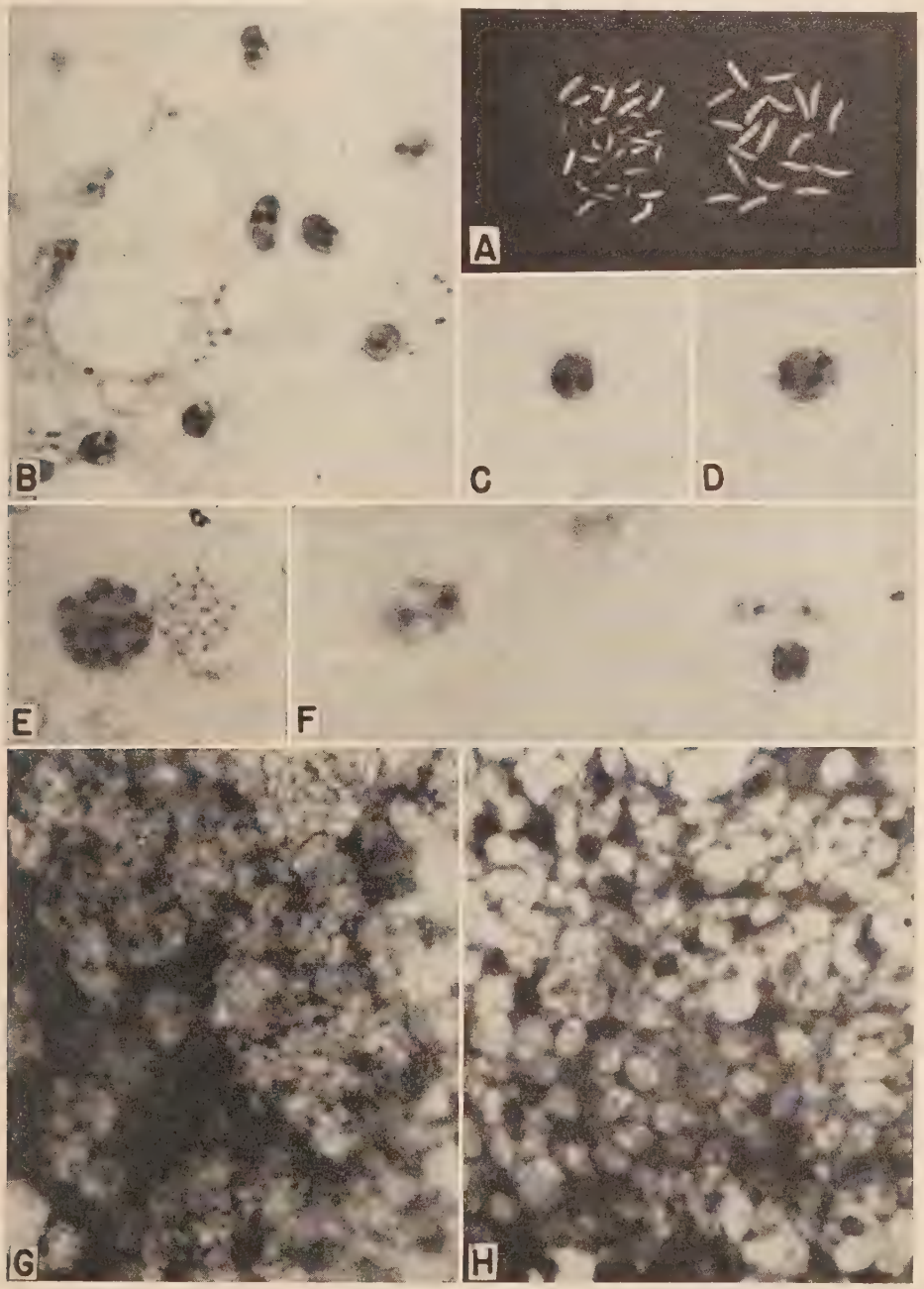
D, Four-nucleate schizont of *N. destructor* (×2000).

E, Pansporoblast (left) and packet of mature spores (right) of P. californica (×2000).

F, Two sporonts and a schizont (lower right) of *N. destructor* (×2000).

G, Smear of tuberworm tissue showing spores of *P. californica* (×1450).

H, Smear of tuberworm tissue showing spores of *N. destructor* (×1450).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SOME ADDITIONAL OBSERVATIONS ON THE MORPHOLOGY OF *DENDROUTERINA BOTAURI* RAUSCH, 1948 (CESTODA: DILEPIDIDAE).

ROBERT RAUSCH*

The scolex has never been described for cestodes of the genus *Dendrouterina* Fuhrmann, 1912. The two species belonging to this genus, *D. herodiae* Fuhrmann, 1912, and *D. botauri* Rausch, 1948, were described from incomplete material. Because of the lack of a scolex, this genus was only tentatively placed by Fuhrmann (1912) in the family Dilepididae.

Persistent collecting during the spring migration of 1948 made possible the examination of a number of bitterns, *Botaurus l. lentiginosus* (Montagu). A single bird, collected on May 5, 1948, at Madison, Wisconsin, was found to be infected with two specimens of *Dendrouterina botauri*. Observations on these entire worms have allowed for the completion of the generic diagnosis as well as for a completion of the specific diagnosis of *D. botauri*.

Genus *Dendrouterina* Fuhrmann, 1912

Diagnosis: Dilepididae. Scolex well developed; rostellum armed with 2 rows of hooks. Genital ducts dorsal to longitudinal excretory canals. Genital pores unilateral. Testes numerous, situated posterior to female genital organs. Uterus highly branched, with lateral branches passing dorsal to ventral longitudinal excretory canal on poral side, and ventral to it on aporal side. Parasitic in birds.

TYPE SPECIES: *D. herodiae* Fuhrmann, 1912.

Additional details on the morphology of *Dendrouterina botauri* Rausch, 1948 (Figs. 1 and 2)

Scolex well developed, about 165 μ in diameter. Suckers 65 μ in longitudinal diameter. Short rostellum armed with two rows of hooks, 18 in number. Longer hooks 28 μ in length; short hooks 20 μ in length. Guard and blade of hooks nearly of same length. Neck well defined, from 500 to 700 μ in length.

Eggs in gravid segments are surrounded separately by a thin, membranous sac up to 75 μ in diameter. Eggs ovoid to spherical, from 30 to 40 μ in longitudinal diameter; shells very thin. Onchosphere ovoid, about 23 by 36 μ (when living); hooks of onchosphere measure 6 and 13 μ in length. The eggs are usually arranged in a single row along the uterine branches.

Observations made on these specimens substantiate those from the type material, as described (Rausch, 1948). It was noted that in even the most terminal gravid segments the seminal receptacle persists within the opening at the center of the uterus. Moreover, the gravid uterus is always closed posteriorly, and is never "hufeisenfoermig" as was described by Fuhrmann (1912) for *Dendrouterina herodiae*. The uterus in *D. botauri* is seen in post-mature segments surrounding the ovary, vitelline gland, and seminal receptacle, at which time a single anterior and two posterior enlargements are visible. Lateral branches slowly develop from these enlargements, but at no time is the early gravid uterus net-like, as it was described for *D. herodiae*.

Study of the complete cestode has indicated that the genus *Dendrouterina* should remain in the family Dilepididae.

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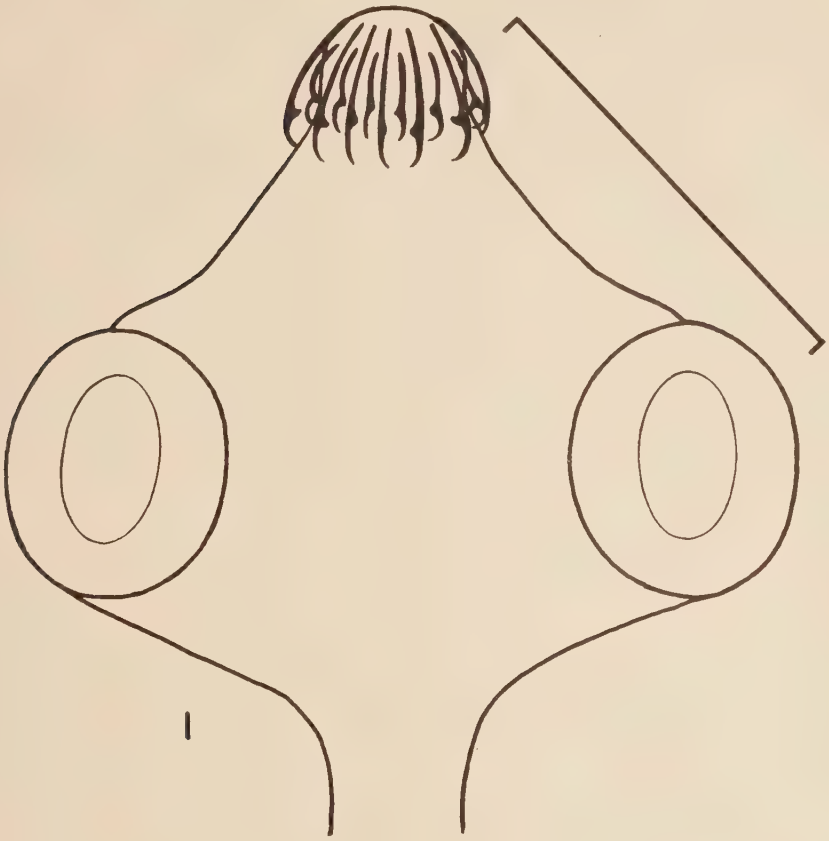

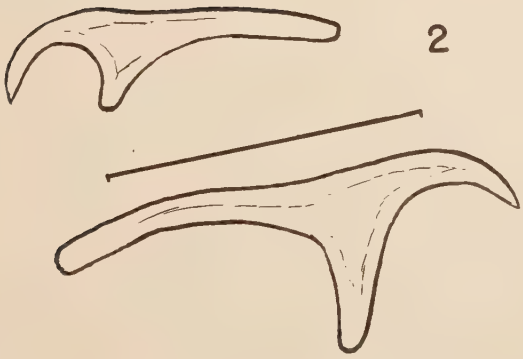

EXPLANATION OF PLATE

FIG. 1. Scolex of *Dendrouterina botauri*. Scale has a value of 100 μ.
FIG. 2. Rostellar hooks of *Dendrouterina botauri*. Scale has a value of 20 μ.

A slide bearing the rostellar hooks of *Dendrouterina botauri* has been deposited in the Helminthological Collection of the U. S. National Museum.

References

Fuhrmann, O. 1912 Ergebnisse der mit Subvention aus der Erbschaft Treitl unternommenen zoologischen Forschungsreise Dr. Franz Werner's nach dem aegyptischen Sudan und Nord-Uganda. XXI. Vogelcestoden. Sitzungsb. K. Akad. Wiss. Wien, Math.-Naturw. Klasse, **121:** 181–192.

Rausch, R. 1948 *Dendrouterina botauri* n. sp., a cestode parasitic in bitterns, with remarks on other members of the genus. Amer. Midl. Nat. **39:** 431–436.

OBSERVATIONS ON BLOOD PARASITES OF BIRDS IN SOUTH CAROLINA

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As part of comprehensive investigations of human malaria in an area adjacent to the Santee Reservoir, South Carolina, a large number of anopheline mosquitoes were collected and dissected during 1945, 1946, and 1947. In discussing results of early phases of this work Sabrosky, et al., (1946) reported the finding of considerable numbers of natural *Plasmodium* infections in salivary glands of both *Anopheles quadrimaculatus* Say and *Anopheles crucians* Wied. Since human malaria is currently at a very low point in this area, and protozoologists are not yet able to differentiate between human and avian malaria sporozoites, the question has arisen as to the identity of the sporozoites found. Experimentally, anophelines have been proved capable of transmitting certain avian malarias (Coggeshall, 1940, 1941; Haas and Akins, 1947; Hurlbut and Hewitt, 1941, 1942; Mayne, 1928). Little is known, however, of their importance in the natural transmission of the common bird malarias. The studies reported herein were undertaken to provide data on the status of avian malaria in the Santee Reservoir area as a contribution toward the solution of this problem.

That bird malaria occurs in the general region in which these studies were made was shown by the work of Thompson (1943) in Georgia, approximately 200 miles distant. To determine the year-around incidence of malaria in the local birds of the Santee Reservoir area, a winter survey was carried out from December 1946 to mid-February 1947, and a summer survey from mid-July to mid-November 1947. Blood slides from a total of 323 English Sparrows, *Passer domesticus domesticus* (L.), and from sixty-four various other birds were studied during the two surveys. Thin smears were prepared in the field and later stained with Giemsa 1:10 for thirty minutes in the laboratory. Slides were examined for at least ten minutes before being considered negative.

During the winter survey one smear from each of 242 birds was examined. The results are given in Table 1. An additional twenty-one birds were trapped for experimental purposes, and repeated blood examinations from them were made. These findings also are included in Table 1 under a separate heading.

It will be noted that 217 English Sparrows were surveyed and that nine individuals or 4.1 percent were positive for avian *Plasmodium*. Three individuals or 12.0 percent of the twenty-five birds of other species examined were positive. Thus, a total of twelve birds of the 242 surveyed was infected with malaria to give a combined positive malaria infection rate of 4.9 percent.

Repeated smears were made from the twenty-one captured birds. One positive was found in the first series, two more in the second for a total of three or 14.3 percent positive.

The summer survey was carried on in the same manner as the winter survey,

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and consisted primarily of a study of 100 English Sparrows. In addition twenty-four birds distributed among the following species were included as indicated: Brown Thrasher, *Toxostoma rufum* (L.)—1; Eastern Field Sparrow, *Spizella pusilla pusilla* (Wils.)—1; Eastern Mockingbird, *Mimus polyglottos polyglottos* (L.)—4;

TABLE 1.—*Blood parasites and bird hosts, winter and spring 1946–1947, Santee Reservoir area, South Carolina*

	Common Name of Bird	Scientific Name	*Plasmodium*								*Haemoproteus* Kruse	*Leucocytozoon* Danilewsky	Avian *Toxoplasma* Nicolle and Manceaux	Negative	Total
			circumflexum Kikuth	*elongatum* Huff	*hexamerium* Huff	*oti* Wolfson	*relictum* (Grassi and Feletti)	*relictum* or *cathemerium*	Number of birds positive	Percent of birds Positive					
Examined Once	English Sparrow	*Passer domesticus domesticus* (L.)	1[1]	1			3[1]	4[2]	9	4.1		2[3]	8[4]	200	217
	Crow	*Corvus brachyrhynchos brachyrhynchos* Brehm.									1			0	1
	Cardinal	*Richmondena cardinalis cardinalis* (L.)						1	1	50.0				1	2
	Eastern Field Sparrow	*Spizella pusilla pusilla* (Wils.)									1			0	1
	Eastern Vesper Sparrow	*Pooecetes gramineus gramineus* (Gmel.)												2	2
	Hawk	Undetermined												1	1
	Song Sparrow	*Melospiza melodia melodia* (Wils.)			1				1	33.3				2	3
	White-throated Sparrow	*Zonotrichia albicollis* (Gmel.)												5	5
	Unidentified	.....			1				1	10.0				9	10
		TOTALS	1	1	2		3	5	12	4.9	2	2	8	220	242
Examined More Than Once	English Sparrow	*Passer domesticus domesticus* (L.)					1[5]		1	16.7				5	6
	Barred Owl (Probably Northern)	*Strix varia* (probably *varia* Barton)				1[6]			1	100.0	1[6]	1[6]		0	1
	White-throated Sparrow	*Zonotrichia albicollis* (Gmel.)			1[7]		1[7]		1	7.1	5[8]	7[9]		4	14
		TOTALS			1	1	2		3	14.3	6	8		9	21

[1] Total includes one probable.
[2] Total includes one (?).
[3] *Leucocytozoon* (?) from bird with Avian *Toxoplasma* and one with *P. elongatum*.
[4] One infection from bird with *Leucocytozoon* (?).
[5] Mixed infection of *Plasmodium* (?).
[6] Mixed infection of three genera.
[7] Mixed infection of *hexamerium* (?), *relictum*, and *Leucocytozoon*.
[8] Two infections mixed with *Leucocytozoon*.
[9] Two infections mixed with *Haemoproteus* and one with *Plasmodium*.

Eastern Vesper Sparrow, *Pooecetes gramineus gramineus* (Gmel.)—1; Hawk, species undetermined—1; Least Flycatcher, *Empidonax minimus* (W. M. and S. F. Baird)—1; Loggerhead Shrike, *Lanius ludovicianus ludovicianus* L.—6; Northern Blue Jay, *Cyanocitta cristata cristata* (L.)—1; Palm Warbler, *Dendroica palmarum palmarum* (Gmel.)—1; Pine Warbler, *Dendroica vigorsi vigorsi* (Aud.)—1; Red-

bellied Woodpecker, *Centurus carolinus* (L.)—1; Red-headed Woodpecker, *Melanerpes erythrocephalus* (L.)—1; Redstart, *Setophaga ruticilla* (L.)—1; Savannah Sparrow, *Passerculus sandwichensis savanna* (Wils.)—1; Southern Downy Woodpecker, *Dryobates pubescens pubescens* (L.)—1; and Sparrow, species undetermined—1.

The findings of the summer survey, made largely during the period when maximum human malaria incidence obtains, show a rate of 2 percent or only half that of the winter rate. Of the 124 birds examined, three English Sparrows showed infections; one had *Plasmodium relictum* (?), one either *relictum* or *cathemerium*, and a third *Haemoproteus* sp. In addition, an Eastern Mockingbird showed *Haemoproteus* sp. and *Trypanosoma* sp.

As a result of this study, the following new host records may be added to the list of those reported by Herman (1944): English Sparrow—*Plasmodium* sp. (probably *circumflexum* and *Leucocytozoon* (?); Barred Owl (probably Northern Barred Owl)—*Plasmodium oti*;[1] White-throated Sparrow—*Plasmodium relictum;* Eastern Field Sparrow—*Haemoproteus* sp.; Eastern Mockingbird—*Trypanosoma* sp.

SUMMARY AND CONCLUSIONS

1. Winter and summer avian-malaria blood surveys, with special reference to the English Sparrow, were made in the Santee Reservoir Area, South Carolina. Data from the winter survey show that 4.9 percent of all birds examined and 4.1 percent of English Sparrows were positive for *Plasmodium*. In the summer survey, these rates were 1.6 percent and 2 percent, respectively. A group of captured birds examined on two successive occasions gave a cumulative infection rate of three times that found on initial examination. Thus, it is indicated that avian malaria actually is much more prevalent than shown by the low rates found on the two surveys, and rates for English Sparrows are lower than for the bird population in general. Doubtless these would have been much higher had subinoculative methods been used.

2. Species of avian *Plasmodium* found in the area are: *circumflexum*,[2] *elongatum, hexamerium, oti,* and *relictum*. In addition, representatives of the following other genera of blood parasites were encountered: *Haemoproteus, Leucocytozoon, Trypanosoma,* and avian *Toxoplasma*.

3. New host records for blood parasites of birds include: *Plasmodium* sp. probably *circumflexum* from the English Sparrow, *Plasmodium oti* from the Barred Owl, *Plasmodium relictum* from the White-throated Sparrow, *Haemoproteus* from the Eastern Field Sparrow, *Leucocytozoon* (?) from the English Sparrow, and *Trypanosoma* from the Eastern Mockingbird.

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[1] *Plasmodium oti* synonymous with *Plasmodium hexamerium* (?) (Manwell, personal communication).

[2] After the completion of this survey, a laboratory bird was infected with *circumflexum* by subinoculation from a White-throated Sparrow.

References

Coggeshall, L. T. 1940 The infection of *Anopheles quadrimaculatus* with a monkey malaria parasite, *Plasmodium cynomolgi,* and with an avian parasite, *Plasmodium lophurae.* J. Parasit. **26** (supp.) : 44–45.

————1941 Infection of *Anopheles quadrimaculatus* with *Plasmodium cynomolgi,* a monkey malaria parasite, and with *Plasmodium lophurae,* an avian malaria parasite. Am. J. Trop. Med. **21:** 525–530.

Haas, V. H., and Akins, H. 1947 Transmission of *Plasmodium gallinaceum* by *Anopheles quadrimaculatus.* J. Nat. Malaria Soc. **6:** 244–245.

Herman, C. M. 1944 The blood Protozoa of North American birds. Bird Banding **15:** 89–112.

Hurlbut, H. S., and Hewitt, R. 1941 Sporozoites of *Plasmodium lophurae,* an avian malaria parasite, in *Anopheles quadrimaculatus.* Pub. Health Rep. **56:** 1336–1337.

————1942 The transmission of *Plasmodium lophurae,* an avian malaria parasite, by *Anopheles quadrimaculatus.* Pub. Health Rep. **57:** 1891–1892.

Mayne, B. 1928 Anopheline Mosquitoes as hosts for parasites of bird malaria. Ind. J. Med. Res. **16:** 557–558.

Sabrosky, C. W., McDaniel, G. E., and Reider, R. F. 1946 A high rate of natural *Plasmodium* infection in *Anopheles crucians.* Science **104:** 247–248.

Thompson, P. E. 1943 The relative incidence of blood parasites in some birds from Georgia. J. Parasit. **29:** 153–155.

FRAGMENTARY STUDIES ON THE LIFE HISTORY OF THE CESTODE *MESOCESTOIDES LATUS*

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The genus *Mesocestoides* Vaillant, 1863, comprises a small, homogeneous group of cyclophyllidean tapeworms. They are common in most carnivorous mammals and birds in Europe, Asia, and Africa, and in North America are occasional in carnivorous mammals and man (Chandler, 1942a). Species are differentiated with difficulty; some authorities hold that there are only three species, others that there are many species. The life cycle was first postulated by Leuckart, who stated that worms known as *Tetrathyridium* Rudolphi from lizards were probably the larvae of *Mesocestoides litteratus* (Batsch) of foxes. The first controlled experiments were reported by Henry (1927), who demonstrated the second half of the life cycle.

The work herein reported consists of unsuccessful attempts to elucidate the first half of the life cycle of *M. latus* of the opossum, together with some incidental observations. My thanks are extended to Dr. Asa C. Chandler, who directed the work.

HISTORICAL

The present review of literature has been selected to include experimental work on only the first part of the life cycle of *Mesocestoides*. For a complete summary of work on this group, see Witenberg (1934).

Several names have been applied to larvae of *Mesocestoides* which have been found in one species of frog and in many species of reptiles, birds, and mammals. The earliest applicable name is *Tetrathyridium* Rudolphi, 1819 (page 514) which has page priority, as well as clearer applicability, over *Dithyridium* Rudolphi, 1819 (page 599). It seems best to use "tetrathyridium" as an English word to designate the second stage larva of *Mesocestoides*. The worm is more or less flattened, elongate when fully grown, unsegmented, with a solid body and an invaginated scolex lacking a rostellum, but bearing four large suckers, each with a slit-like opening. Complete lists of hosts were given by Witenberg (1934) and Hughes, Baker, and Dawson (1941).

Neumann (1896) fed gravid segments of *Mesocestoides lineatus* to four dogs and recovered immature *Mesocestoides* adults on the 62nd day from one of them. He used no controls, and his animals were neither laboratory raised nor laboratory housed during the experiments.

Henry (1927) infected cats with adult *Mesocestoides lineatus* by feeding them naturally-occurring tetrathyridia from cats. He attempted, without success, to infect dogs, cats, rabbits, guinea pigs, sheep, white mice, chickens, ducks, geese, pigeons, frogs, flies (*Musca domestica* and *Calliphora* sp. adults), and cockroaches by feeding them gravid segments of *Mesocestoides lineatus* from a cat.

Schwartz (1927) could not infest dogs and cats with gravid segments of *Mesocestoides* sp. from a dog and a cat. Schultz (1927) found tetrathyridia in one of

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twelve house mice fed ripe segments of *Mesocestoides* sp. Any precautions to eliminate natural infection were not mentioned in his report. Joyeux, Baer, and Martin (1933) found tetrathyridia in both of two cats fed gravid segments of *M. lineatus*. However, no precautions were taken to be sure that the cats were not previously infected. Markowski (1934) could not infect white mice, a rook, or a crow by feeding ripe segments of *M. lineatus*. Witenberg (1934) could not infect white mice, lizards, house flies, or cockroaches by feeding ripe segments of *M. lineatus* forma *lineatus*. Witenberg noted that onchospheres encased in the parauterine organ of gravid segments remained viable for eight days in physiological saline.

THE PROBABLE LIFE CYCLE OF MESOCESTOIDES

It seems reasonable to assume that the life cycles of the several species of *Mesocestoides* are similar because the adult tapeworms are so much alike that they are distinguished with difficulty. According to the postulates of Henry (1927), Witenberg (1934), and the writer, the life history of *Mesocestoides* may be postulated as follows:

The eggs are eaten by a suitable invertebrate in which the onchosphere develops into a cysticercoid. If the invertebrate host is eaten by any amniote vertebrate, the cysticercoid develops into a mature larva, a tetrathyridium, in the body cavity, liver, mesentery, peritoneum, heart muscle, or subcutaneous tissue, reaching these sites by active migration. If this second intermediate host is eaten by a tetrapod vertebrate other than a suitable definitive host, the tetrathyridium re-encysts in the body cavity or mesentery (See Joyeux and Baer, 1933), but if eaten by the definitive host it develops to maturity directly in the intestine.

Two alternative hypotheses are open to consideration:

(1) The invertebrate first intermediate host step might be omitted. This hypothesis is supported by the uncontrolled experiments reported by Neumann (1896), Joyeux, Baer, and Martin (1933), and Schultz (1927), mentioned above. The validity of these experiments may justifiably be questioned in view of the numerous controlled experiments performed by Henry (1927), Schwartz (1927), Joyeux and Baer (1933), Markowski (1934), and Witenberg (1934), on numerous species of reptiles, birds, and mammals, all of which were negative and indicated that direct infection of vertebrates by the eggs of *Mesocestoides* is not possible.

(2) The vertebrate second intermediate host might be only a convenient but not obligatory stage, inserted into the life cycle only if the invertebrate first intermediate host was eaten by a vertebrate in which the tapeworm could not mature but serving as a good means of access to the carnivorous definitive hosts. The common occurrence of tetrathyridia in the same animals that also harbor the adult worm was emphasized by Witenberg (1934); he regarded this fact as militating against this hypothesis.

That the first intermediate hosts are widely distributed invertebrates is indicated by the wide range of *Mesocestoides*. That the first intermediate hosts are terrestrial is indicated by the following facts: adult *Mesocestoides* are found only in strictly terrestrial birds and mammals, although a few, such as racoons and opossums, sometimes eat shellfish and fish. Within the family *Mustelidae,* no forms of *Mesocestoides* have been found in the semi-aquatic minks and otters; adult *Mesocestoides* have been found in the strictly terrestrial civetcats, skunks, and *Myonax;*

both tetrathyridia and adult *Mesocestoides* are found in the strictly terrestrial badgers, weasels, martens, and polecats. The common occurrence of *Mesocestoides* in such an arid country as Palestine argues against an aquatic or amphibious intermediate host.

It seems probable that the invertebrate in question is a predaceous form which would eat the large piece of mucoid flesh represented by an entire segment, or at least the heavy-walled parauterine organ. On the other hand, it is unlikely, though not entirely excluded from consideration, that the segments habitually disintegrate on the ground and that the small, thin-shelled eggs are eaten by a small form that feeds on decaying animal matter.

That the invertebrate in question is an arthropod, rather than an annelid or mollusc seems probable from an inspection of the list of hosts of tetrathyridia as given by Witenberg (1934). Many of these hosts (*e.g.* house mice, marmots, monkeys, bats, lizards, small snakes) seldom or never eat earthworms or molluscs but subsist largely on arthropods and vegetable matter. Neither could these forms have acquired the tetrathyridia by eating another vetebrate because they seldom or never eat other vertebrates. Witenberg (1934) and Markowski (1934) both suggested dung bettles of the genus *Geotrupes*.

The possibility that the invertebrate in question is a parasite such as a flea or a louse seems unlikely as a corollary to the necessity of a second intermediate host. If the first intermediate host were a flea or louse living on, or in the home of, the opossum or other definitive host, how could the small vertebrate become infested, and why would the tetrathyridium be limited to insectivorous forms and the adult tapeworm to carnivorous forms? The same argument precluded the probability of a mite or other small arthropod that would be eaten with vegetation being involved. It seems most likely that the invertebrate in question is a widely distributed, fairly large-sized, carnivorous, terrestrial arthropod.

INFECTION EXPERIMENTS

Material used for the infection experiments was all obtained from a single opossum (*Didelphis virginianus*). The animal was captured in Houston, Texas, and kept in a slat-bottomed cage over a pan of water for the duration of the experiments. It was fed on dried commercial dog food and water, varied occasionally with horse meat. Segments were passed the day after the animal was captured and nearly every day for eight months thereafter. Some of these gravid segments, and some sections of strobila passed on three occasions, were stained and mounted. They appeared identical in all respects with the two complete specimens of *Mesocestoides latus* Mueller, 1927, which had been studied by Chandler (1946).

Each day gravid segments were removed from the pan, washed in physiological saline solution, and stored at room temperature in a covered dish. The bottom of the dish was covered with filter paper moistened with physiological saline. For each species of experimental animal, some of the segments used were fresh, some one or two days old, and some three to six days old. Time on each experiment was reckoned from the day the infective meal was first given. Many additional animals were fed, but died prior to the fourteenth day. They were dissected and found negative, but are not reported here. Two cysts, which were apparently very immature cysticercoids, were found in separate pill-bugs (*Armadillidium vulgare*); but their scar-

city indicated that they were not experimentally produced. The white mice were laboratory-raised, and were provided by Dr. W. M. Fisher of Baylor Medical School. Infection experiments comprise Table 1.

TABLE 1.—*Infection experiments performed on* Mesocestoides latus. *All results were negative.*

Experimental animal	Feeding of gravid segments	Number dissected	Time in days
Flatworms:			
Unidentified triclad turbellarian	Plain, entire.	2	49
Earthworms:			
Lumbricus sp.	Dissected and mixed with soil, and entire, on surface.	2	21
Helodrila sp.		1	21
Crustacea:		5	25–28
Armadillidum vulgare	Entire. Many eaten.	34	36–44
		1	53
Asellus sp.	Entire. Few eaten.	4	16
[1]*Stygobromus bifurcus*	Entire. Many eaten.	21	14–19
[2]*Cambarus diogenes*	Entire. Many eaten.	6	17–26
Myriapods:			
Polyxenus sp.	Entire. Many eaten.	1	32
Polydesmidae unidentified sp.	Entire. Many eaten.	4	38
Scoplopendridae unidentified sp.	Entire.	3	35–39
Julidae unidentified sp.	Entire. Many eaten.	1	23
Springtails:			
Entomobryidae unidentified sp.	Entire.	2	38–39
Orthoptera:			
Blatta orientalis	Entire. Many eaten.	4	20–43
Periplaneta americana	Entire. Many eaten.	5	20–28
Tetrigidae unidentified sp.	Dissected and entire in bran mash; some eaten.	4	22–35
Locustinae unidentified sp.		1	32
Decticinae unidentified sp.		1	32
Gryllus sp.		1	14
Flies:			
Musca domestica	Fed only to larvae. Many entire segments eaten.	7 larvae, 6 pupae, 1 adult	15–22
Beetles:			
Carabaeidae unidentified sp. adults	Entire. Few eaten.	2	32–35
Alaus sp. larvae	Entire. Few eaten.	4	35–39
Tenebrionidae unidentified sp., burrowing larva of a darkling ground beetle	Entire and dissected in and on earth. Few eaten.	1	38
Tenebrio molitor adults	Dissected and entire mashed into pieces of apple. Few eaten.	7	29–31
Tenebrio molitor larvae		19	29–31
Tribolium confusum adults		3	31
[2]*Canthon laevis* adults	Dissected and entire stirred into balls of cow manure. Many eaten.	5	32–59
[3]*Aphodius fimetarius* adults		9	25–37
[3]*Geotrupes blackburni* adults		3	18–22
[3]*Hister* sp. adults		2	28
[4]*Phocera* sp. adults		1	18
Phyllophaga sp. larvae	Dissected and entire stirred into dirt and manure mixture.	5	25–43
[5]*Pelidnota punctata* larvae		10	41–100
Ants:			
[6]*Solenopsis geminata*	Entire, plain. Few eaten.	21	20
Mollusks:			
[7]*Euconulus charsinus trochulus*	Dissected mixed with corn meal and plain entire. Smaller snails ate former; larger snails ate many of latter.	4	33
[7]*Oligyra orbiculata*		1	33
[7]*Stenotrema monodon aliciae*		17	28–33
[7]*Mesodon thyroidus*		18	25–39
[7]*Zonitoides arboreus*		20	34–37
[7]*Phylomycus carolinensis*		2	28–36
White mice:		5	34–42
Mus musculus	Each fed 3 entire.	3	59–60
		1	87

[1] Identified by Mr. Clarence R. Shoemaker of the U. S. Nat. Mus.
[2] Identified by Dr. H. H. Hobbs, Jr., of the U S. Nat. Mus.
[3] Identified by Dr. H. J. Reinhard of Texas A. and M. College.
[4] Identified by Mr. C. P. Read of Rice Institute.
[5] Identified by Dr. W. H. Anderson of the U. S. Nat. Mus.
[6] Identified by Dr. M. R. Smith of the U. S. Nat. Mus.
[7] Identified by Dr. J. P. E. Morrison of the U S. Nat. Mus.

DISCUSSION OF EXPERIMENTAL RESULTS

Negative results with mice serve to confirm the work of Henry, Schwartz, Markowski, and Witenberg, which had indicated that an invertebrate first intermediate

host was necessary. Negative results with 36 species of invertebrates cast doubt upon the ability of these species to act as intermediate hosts.

It is suggested that other families and genera of ants, beetles, roaches, and terrestrial isopods should be tested.

DESCRIPTION OF ADULT

The adult *Mesocestoides latus* is a narrow tapeworm; the anterior segments are approximately square and the posterior segments bead-shaped. There are 500 to 1,000 segments; entire worms with gravid segments are 100 to 123 cm. long. The anatomy has been described in detail by Mueller (1927, 1928, 1930) and Chandler (1942, 1946), but a few points remain to be clarified. The writer finds 51 to 59 testes; cirrus pouch 146 to 188 μ long; egg capsule 602 to 766 μ long by 465 to 565 μ wide in four complete specimens from Houston opossums. Nine opossums were examined, from Harris, Montgomery, and Brazoria Counties, Southeast Texas. Two were infested with *Mesocestoides latus*.

BEHAVIOR AND BIOLOGY OF SHED SEGMENTS AND OVA

The gravid segments when passed in the feces of the opossum migrate actively out of the fecal mass. This seems to indicate that the first intermediate host is not exclusively, if at all, coprophagous. Segments removed from still-worm stools were observed to move fairly rapidly for a few minutes, traversing at least three inches. They crawled along a dry, horizontal or vertical glass surface or on moistened filter paper. Some of them moved more than an inch from 15 minutes after passage until two hours later. Thereafter they did not move. None of the segments crawled under the paper.

Other freshly-passed segments were placed on damp sand at a temperature of 23° C. beside an open window. They moved slightly, horizontally, for the first hour, but showed no tendency to move under the grains of sand.

Actively crawling, freshly-passed segments were placed on a horizontal damp glass surface. A 150-watt light was placed to shine horizontally across the glass plate, the room being otherwise dark. No tendency to crawl either toward or away from the light was noted; the crawling was apparently aimless.

Two experiments were performed in order to test the viability of the ova. A group of freshly-passed segments was placed on a piece of damp filter paper lying on an inch of damp soil in a finger bowl. The bowl was in an unheated room beside an open window. The weather was foggy, the room temperature 23° C. for the duration of the experiment. Five hours after the start of the experiment the filter paper appeared dry; it was turned over. Twenty-seven hours after the start the segments appeared dry and desiccated; but the onchospheres were viable when the eggs were dissected out of one segment and placed in physiological saline under a cover slip. Forty-eight hours after the start of the experiment the remaining segments appeared very dry and desiccated. The thickest segment was dissected, but no viable onchospheres could be found within it. Onchospheres were considered viable if movement could be seen within the eggs.

Another group of freshly-passed segments was placed on a piece of filter paper and kept moistened with physiological saline on the bottom of a covered dish. One segment was dissected each day; viable onchospheres were found up to and including the ninth day. On the tenth day no further movement of onchospheres was seen.

DESCRIPTION OF ONCHOSPHERE

Within the egg capsule of each gravid segment of *Mesocestoides latus* are 100 to 200 eggs. Each egg has a single, rather thin, transparent shell, closely investing the mature hexacanth onchosphere. The living, liberated onchosphere is usually ellipsoid, but sometimes ovoid with the large end anterior. In the ten specimens measured, the total length varied from 30 to 40 μ and the length of the median hooks from 16 to 17 μ. The four lateral hooks are very slightly shorter than the median pair.

Onchospheres were freed from the egg shell by placing them in a weak aqueous solution of pancreatic extract under a cover slip. In 10 to 15 minutes some of the shells ruptured and their onchospheres began to crawl out. By 30 minutes all of the shells were partially dissolved away, and had ruptured, leaving the onchospheres free.

TETRATHYRIDIA

The writer's knowledge of the tetrathyridium stage of *Mesocestoides* is based upon two collections:

(1) Harwood (1932) recorded finding "Cysticercus sp." three times in lizards at Houston—twice in *Leiolepisma laterale* and once in *Eumeces fasciatus*. One specimen of this collection, stained and mounted in balsam, still remains in the Rice Institute collection, where it was studied by the writer.

The cyst is 0.7 mm. long and contains a completely inverted, partially-developed scolex 264 μ wide, bearing four suckers 83 to 99 μ long, with slit-like openings. The scolex is attached to the parenchyma by a few strands of tissue (fig. 2) and most of the body of the worm is filled with loose parenchyma. In my opinion, it is an immature tetrathyridium of *Mesocestoides variabilis* Mueller because the measurements fit the scolex of that species better than that of any other known from Texas.

(2) On July 7, 1941, the writer removed more than 50 tetrathyridia from the liver of a white-footed mouse, *Peromyscus boylii boylii*. The mouse had been trapped by Miss Jean T. Boulware, and was identified by Mr. Joseph S. Dixon, then of the U. S. Fish and Wildlife Service. The location was Miguel Meadow, Yosemite National Park, California.

Twenty-seven stained and mounted specimens were studied in detail. Of these, five had a secondary evagination of the scolex (as in fig. 3). The worms varied in length from 2 to 5 mm.; the suckers were 182 to 237 μ long; the scolices were 347 to 529 μ wide (only 16 measured for the last two characters). The small size of the scolices seems to indicate that these larvae are *M. variabilis* or *M. kirbyi* Chandler.

SUMMARY

It is postulated that the first intermediate host of *Mesocestoides* is a terrestrial carnivorous arthropod and that a second intermediate host, a vertebrate, is required before completion of the life cycle.

Gravid segments of *Mesocestoides latus* were fed to white mice, and to 531 individuals of 36 species and four phyla of invertebrates, but no infection resulted. 263 of the invertebrates were dissected after 14 to 100 days.

Second-stage larvae (tetrathyridia) of *Mesocestoides* are recorded from a white-footed mouse in California and a lizard in Texas.

References

Chandler, A. C. 1942 *Mesocestoides manteri* n. sp. from a lynx, with notes on other North American species of *Mesocestoides*. J. Parasit. **28**: 227–231.

———1942a First record of a case of human infestation with tapeworms of the genus *Mesocestoides*. Amer. J. Trop. Med. **22**: 493–496.

———1946 Observations of the anatomy of *Mesocestoides*. J. Parasit. **32**: 242–246.

Harwood, P. D. 1932 The helminths parasitic in the Amphibia and Reptilia of Houston, Texas, and vicinity. Proc. U. S. Nat. Mus. **81**: 1–71.

Henry, A. 1927 *Tetrathyridium et Mesocestoides*. Rec. Méd. Vét. **103**: 147–152.

Hughes, R. C., Baker, J. R., and Dawson, C. B. 1941 The tapeworms of reptiles. Part 1. Amer. Midl. Nat. **25**: 454–468.

Joyeux, C., and Baer, J. G. 1933 Le réencapsulement de quelques larves de cestodes. C. R. Acad. Sci. Paris **197**: 493–495.

———, ———, and Martin, R. 1933 Sur le cycle évolutif des *Mesocestoides*. C. R. Soc. Biol. Paris **114**: 1179–1180.

Markowski, S. 1934 Beitrag zur Kenntnis der Entwicklung der Larve *Tetrathyridium variabile* (Diesing 1859). Mém. Acad. Polon. Sci. Lett., Math. Nat., B: Sci. Nat. 1934: 43–51.

Mueller, J. F. 1927 Two new species of the cestode genus *Mesocestoides*. Trans. Amer. Micr. Soc. **46**: 294.

———1928 The genus *Mesocestoides* in mammals. Zool. Jahrb. Syst. **55**: 403–418.

———1930 Cestodes of the genus *Mesocestoides* from the opossum. Amer. Midl. Natur. **12**: 81–90.

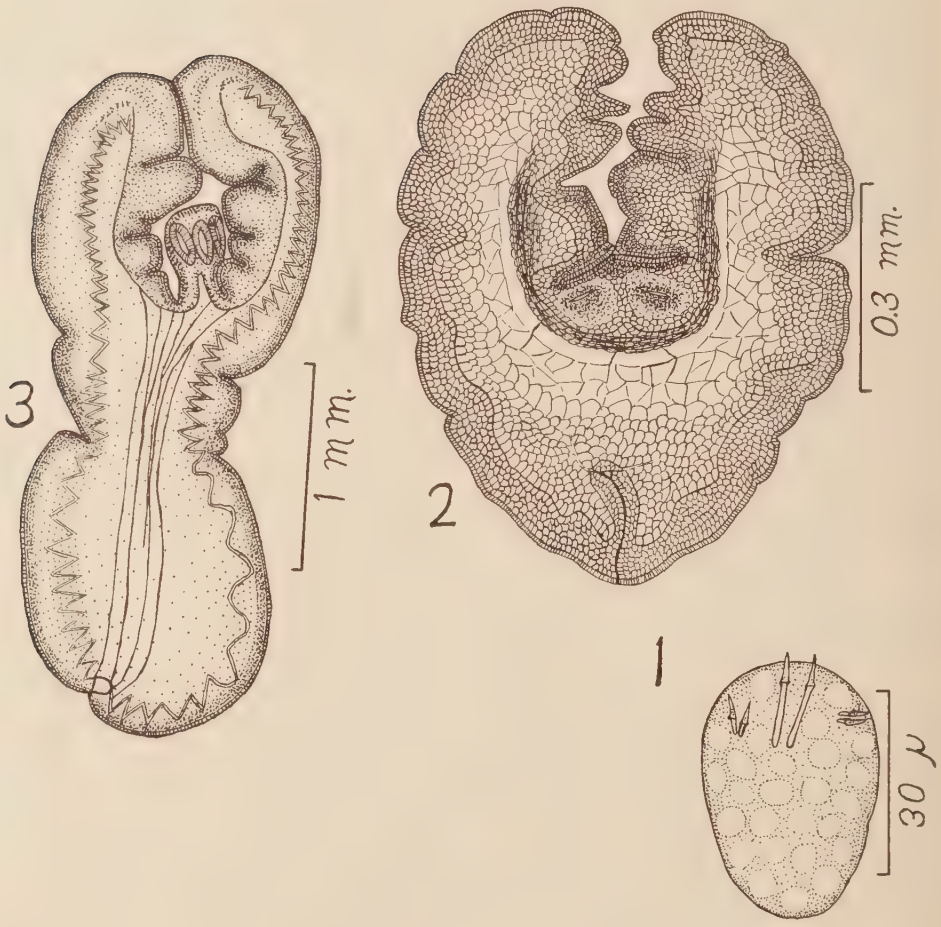
Neumann, L. G. 1896 Notes sur les téniadés du chien et du chat. Sur le genre *Mesocestoides*. Mém. Soc. Zool. France, **9**: 171–184.

Rudolphi, C. A. 1819 Entozoorum synopsis, cui accedunt mantissa duplex et indices locupletissimi. 1–811. Berlin.

Schultz, R. E. 1927 The twentieth helminthological expedition in U.S.S.R. in Novotscherkassk. Results Twenty-eight Helminthological Expeditions in U.S.S.R. 187–191. Moscow.

Schwartz, B. 1927 The life history of tapeworms of the genus *Mesocestoides*. Science **66**: 17–18.

Witenberg, G. 1934 Studies on the cestode genus *Mesocestoides*. Arch. Zool. Ital. **20**: 487–508.



Explanation of Plate

All figures were drawn with the aid of a camera lucida.

Fig. 1. Living onchosphere of *Mesocestoides latus*.

Fig. 2. Immature tetrathyridium from a lizard. Probably *Mesocestoides variabilis*.

Fig. 3. Full-grown tetrathyridium from white-footed mouse. Probably *Mesocestoides variabilis* or *M. kirbyi*.

EXPERIMENTAL INFECTION OF THE SNAIL *AUSTRALORBIS GLABRATUS* WITH THE TREMATODE *SCHISTOSOMA MANSONI* AND THE PRODUCTION OF CERCARIAE*

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For the past three years there has been in progress in this Laboratory a study of the effect of drugs on experimental infections of *Schistosoma mansoni* in mice. To supply fresh cercariae continuously for this purpose a snail colony of *Australorbis glabratus* has been maintained. In keeping this colony replenished, some statistical and quantitative data have been gathered relative to the infection in snails and to the cercariae produced under laboratory conditions. These data will be presented under the following headings: (1) The percentage of snails that becomes infected on exposure to miracidia under a uniform set of conditions; (2) The time at which half the miracidia die under these conditions; (3) The production of cercariae by infected snails under specific controlled conditions; (4) The time at which half the infected snails die; (5) The time at which half the cercariae die under a few specific conditions. The time at which half of each of these populations dies is not referred to as a half life time because some of them do not follow typical exponential die-away curves.

The Infection of Snails. The initial stock of infected snails was generously furnished by Dr. H. W. Stunkard of New York University. These were progeny of Puerto Rican *Australorbis glabratus* and were infected with Puerto Rican *Schistosoma mansoni*. The colony has been continually replenished since November, 1944 by infecting descendents of this stock with miracidia hatched from eggs passed in feces of infected mice or hamsters. About eight fresh fecal pellets from infected mice or hamsters were broken up in about 100 ml. of Great Bear Spring water and allowed to stand one to two hours at a temperature of 28°–30° C. With the aid of a dissecting microscope, five to seven miracidia were picked out with a minimum amount of water in a micro-pipette, and transferred to a five ml beaker. Three ml of spring water and a young snail six to eight mm in diameter were added. After about three hours most of these miracidia could no longer be found, but the snails were usually left in these small beakers overnight and then transferred in groups to larger aquaria. Four weeks later these snails were put out individually into five ml beakers with spring water to determine whether they were shedding cercariae. Those not shedding cercariae were set out individually in this way once a week for about six weeks. Those that began to shed cercariae during this period, between four and ten weeks after exposure to miracidia, were counted as infected. Some snails were also exposed to infection with smaller and larger numbers of miracidia.

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TABLE 1.—*Relation between density of exposure to miracidia of* S. mansoni *and percent of snails* A. glabratus *that shed cercariae during the following ten weeks*

Source of infected fecal pellets	Density of exposure; number of miracidia per snail	Total number of snails exposed	Number of snails that begin to shed cercariae during the following weeks after exposure			Total number of snails that produce cercariae by the tenth week after exposure	Percent of snails that produce cercariae by the tenth week after exposure	Remarks
			4–5	6–7	8–10			
Mouse or hamster	1	127	6	3	1	10	8	
Mouse or hamster	5–7	867	260	183	27	470	54	
Mouse	5–7	278	...	...	....	133	48	
Hamster	5–7	553	136	192	6	334	60	14% of the snails died during the ten weeks.

The results are summarized in Table 1. This table shows the number of snails that began to shed cercariae when examined at intervals between four and ten weeks after exposure to miracidia. Even when snail and miracidia are kept closely crowded together as described and with numbers of miracidia up to seven per snail not more than fifty to sixty percent of the exposed snails begin to shed cercariae during the following ten weeks. At first exposures of snails were made to miracidia hatched from eggs passed in either mouse or hamster fecal pellets. Subsequent work was done keeping separate the snails exposed to miracidia from mice and those exposed to miracidia from hamsters. The difference in the percent of snails that become infected from these two sources is slight and probably not significant. Records were kept of the number of snails that died during the period from exposure to miracidia until ten weeks later in only the last series run. This is shown in the fourth row of Table 1. Of 553 snails exposed to miracidia from hamster feces, 14 percent died during the following ten weeks while 60 percent began to shed cercariae. The remaining 26 percent survived but shed no cercariae during the ten week period following exposure. This would indicate that about a quarter of the snails were refractory to infection. It would be worth re-exposing such once-exposed but uninfected snails to find out whether this apparent condition of being resistant is permanent. Hunter, Bennett, Ingalls and Greene (1947) showed that after exposure of *Oncomelania quadrasi* to as many as ten miracidia of *Schistosoma japonicum* no more than a third of the snails surviving ten weeks later were found to be infected.

Survival of miracidia. Four miracidia were set out in each of a number of beakers exactly as described for infection of snails except that no snails were included. These beakers were kept at 24°–26° C in an incubator and the surviving miracidia were counted at two hour intervals. The results are given in Table 2. Zero time is the time the miracidia were pipetted into the five ml beakers of spring water. At this time they could not have been hatched for more than one hour since the suspension of feces in water was not used for this purpose more than an hour after its

TABLE 2.—*Number of freshly hatched miracidia surviving in spring water at 24°–26° C*

Time, Hours	0	2	4	6	8	10	22
Exp. 1	100	99	98	95	53	..	0
Exp. 2	100	100	99	93	56	19	0
Exp. 3	100	100	98	91	70	40	0

preparation. The time at which half the population has died is about eight hours. Maldonado and Acosta-Matienzo (1947), in a recent abstract of work not yet reported in detail, showed that *S. mansoni* miracidia in three hours reached a condition where only half were alive and able to penetrate the snail but all were dead in nine hours. It is particularly interesting to compare their observations with the results reported in Tables 1 and 2 which show that though 98 percent of the miracidia were alive three hours after hatching only about 60 percent of snails exposed to six or seven miracida subsequently shed cercariae.

Liberation of cercariae by snails. For the past three years, generally four or five times each week, counts have been made of the cercariae liberated by the colony of infected snails. These snails had all been exposed to infection with five to seven miracidia by the method described and after waiting to see which would produce cercariae, the infected snails were segregated in aquaria. Cercariae were counted using the method previously described by Schubert (1948).

The results of almost six hundred of these daily counts on infected snails which had been exposed to from five to seven miracidia will be considered in several ways. First is the question as to whether there is, under the rather uniform and unnatural

TABLE 3.—*Average number of cercariae produced per snail per day under the standard conditions described, for successive two month periods*

Two Month Period	1945			1946			1947		
	Number of counts	Average number of snails	Cercariae produced per snail per day	Number of counts	Average number of snails	Cercariae produced per snail per day	Number of counts	Average number of snails	Cercariae produced per snail per day
Jan.–Feb.				42	20	656	40	30	423
Mar.–Apr.				40	42	875	36	49	997
May–June	59	9	714	39	48	879	40	56	928
July–Aug.	43	11	320	42	37	429	32	35	637
Sept.–Oct.	40	24	929	41	41	326	30	17	674
Nov.–Dec.	34	16	761	27	19	209	9	43	599

laboratory conditions of housing the snails, any seasonal variation in average cercarial production. The data averaged over successive two month periods is given in Table 3. No consistently high or low counts are associated with any season though on several occasions long periods of low average counts have occurred. Over this entire period the weighted average number of cercariae liberated per snail per day was 698 and these daily averages fluctuated within a range from the lowest value, 14, to the highest value, 4158. But though these amounts of daily cercarial liberation fluctuate between wide limits and are largely unpredictable as to size, one regularity soon came to be recognized; that the liberation of cercariae on Monday was generally, but by no means always, much higher than on any other day of the week. This is shown in Figure 1 which gives graphically the daily average cercarial emergence per snail over two periods of time, one of which is the period of our minimum, the other the period of our maximum average daily liberation of cercariae per snail. These figures show that frequently emergence, after a rest period of a few days during which snails were not stimulated to liberate cercariae by exposure to light and raised temperature, was higher than after several successive days of such stimulation. Exceptions are, however, numerous. There is for example in the month of April the high emergence on a Friday after four successive days of stimulation. Averaging only the emergence for Mondays over the same period of time

as is covered in Table 3 gives a figure of 1182 cercariae per snail instead of the overall average of 698. This information on the regularities of cercarial liberation is important in chemotherapeutic work where it may be necessary to anticipate suffi-

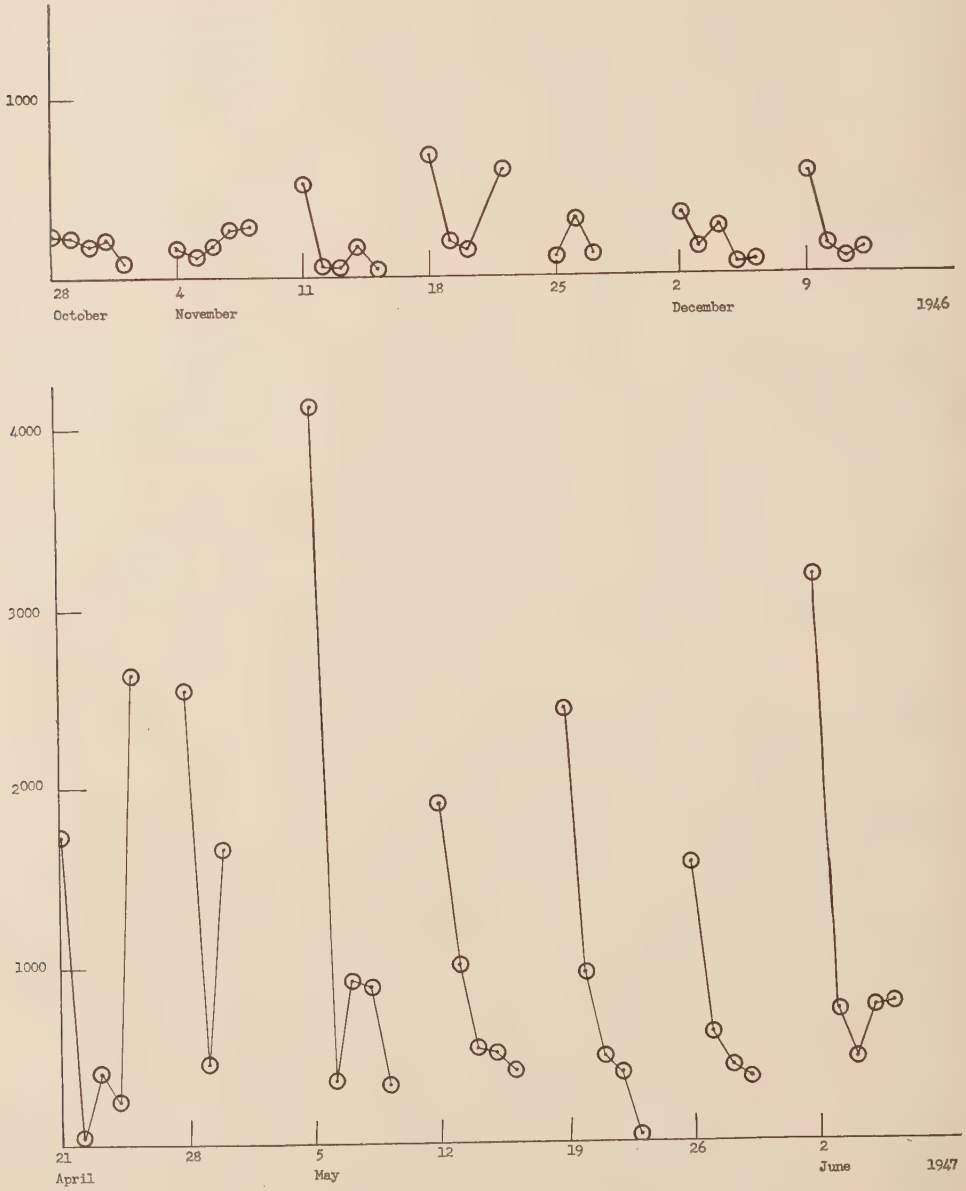

FIG. 1. Daily cercarial production per snail per day during the month of lowest and during the month of highest average production. Ordinates: Number of cercariae produced per snail per day. Abscissae: Days of the counts. The days marked are Mondays.

cient cercarial production for animal exposures. It also raised questions as to the stimulus which induces emergence of cercariae from the snail.

The method adopted to stimulate cercarial shedding by the snails involves warm-

ing the snails with a 100 watt electric light bulb at a distance of about a foot. This supplies light as well as heat and so some experiments were set up to determine whether only one or whether both of these factors was responsible for the stimulation of cercarial production. A group of infected snails was divided at random in each experiment into two equal sub-groups of 10 to 20 snails. Each of these sub-groups was set out separately in a beaker as described before for stimulation of cercarial production but each beaker was covered by a larger inverted beaker, one of which was coated with black light-tight paper. Measurements showed that the temperature of water set up in this way rose to 30° in the undarkened beaker but was

TABLE 4.—*Comparison of cercarial production by snails under light and dark conditions*

9:00 A.M. to 11:00 A.M.				11:00 A.M. to 3:00 P.M.			
Dark		Light		Dark		Light	
Number of snails used	Average no. of cercariae produced per snail	Number of snails used	Average no. of cercariae produced per snail	Number of snails used	Average no. of cercariae produced per snail	Number of snails used	Average no. of cercariae produced per snail
Part A: Snails set out in beakers in an air bath at 30°							
11	1311	11	1033	10	105	10	130
12	183	12	556	11	1344	12	1316
10	695	12	1187	11	1331	12	1308
17	75	17	231	12	1072	11	1604
16	1341	16	2257	10	542	11	1064
16	140	15	625	11	379	10	1161
15	3190	14	2687	11	984	11	1072
14	109	15	516	11	472	10	400
14	170	14	336	13	577	12	1146
13	1878	12	5184	16	82	16	525
21	892	22	2773	15	158	15	1065
23	365	24	750	23	196	20	174
23	523	24	1259	21	244	22	380
23	163	22	271	26	151	23	617
				24	429	23	697
				23	163	22	271
Average	735		1345		434		716
Part B: Snails set out in Erlenmeyer flasks in a water bath at 30°							
22	726	23	771	24	98	21	406
22	290	22	426	22	127	23	216
21	1228	23	1363	21	117	23	267
21	187	19	364	21	448	20	1569
14	41	14	316	20	231	19	737
12	1400	13	1115	12	2020	13	2470
10	149	10	436	14	170	14	323
10	1048	10	858	13	131	13	455
10	105	10	321	12	83	10	432
8	738	10	969	11	109	11	344
8	37	7	175	10	822	10	835
				10	136	10	340
				10	210	10	579
				10	284	10	836
				7	331	9	182
				8	71	8	270
				8	1224	8	783
Average	511		692		340		654

about 2° lower in the darkened beaker. The results of a series of measurements of cercarial production under these conditions on several different groups of snails is summarized in part A of Table 4. The production of cercariae per snail is clearly higher on the average in the beaker to which both light and warmth have access. Yet there was still a possibility that in the beaker covered with black paper not only was light excluded but the rise in temperature might be a little slower. So another series of experiments was set up similar to those described except that the sub-groups of snails were transferred to two 250 ml Erlenmeyer flasks suspended in a water bath kept at 30° C and exposed to normal daylight together with illumination from

a 100 watt electric light bulb. One of the Erlenmeyer flasks was darkened by an opaque coat of black enamel and kept loosely covered with a cotton plug. With this arrangement both the final temperature and the rate of temperature rise were the same for the two sub-groups of snails. The results are summarized in part B of Table 4 and again show that in the illuminated flasks the cercarial production is 35 and 90 percent higher on the average in the sets of experiments run in morning and afternoon. Though this positive effect of light in increasing cercarial production appears in most individual runs, there are a few cases in Table 4 in which the production is higher for the darkened snails. The average values given at the bottom of each group of runs are weighted averages. Brackett (1940) showed that infected *Lymnaea stagnalis* snails kept in darkness produced *Cercaria elvae* readily when the temperature of their environment was raised. Isobe (1923) concluded from experiments on *Schistosoma japonicum* that the temperature of the water is the controlling factor in cercarial production and that lightness and darkness are indifferent factors. Rees (1931) studied the effect of light and darkness on the production of three different species of cercariae. *Lymnaea palustris* and *L. peregra* infected with *Cercaria limbifera* and *C.Z* liberated no cercariae when kept 24 hours in the dark but did produce cercariae when illuminated by daylight. The snail *L. truncatula,* however, liberated larger numbers of *C. cambrensis* in the darkness than it did in the light. The conclusion to be drawn from Table 4 is that although *Australorbis glabratus* infected with *Schistosoma mansoni* does shed cercariae when stimulated by warming to 30° C between the hours 9 A.M. and 3 P.M. even when kept in the dark, they shed more cercariae on the average when simultaneously illuminated.

One other question important enough to deserve some attention was concerned with whether cercariae could be produced from infected snails as readily in the morning as in the afternoon. Data in Table 4 already indicate that this is the case. Further experiments were carried out to find out whether after shedding cercariae in the morning, the same snails would also shed cercariae at noon. Groups of snails were set out as already described from 9:30 A.M. until 11:00 A.M. and then transferred to another beaker containing the same amount of water and left under the warming electric bulb from 11:00 A.M. until 3:00 P.M. The cercariae produced in these two periods of time were separately counted and the results are listed in Table 5. The average values of these two periods are simple averages not weighted with respect to the number of snails in each individual run. More cercariae per snail were produced in the noon period than in the morning period, on the average. But if the production per hour is calculated the figure for the morning period is higher. The average production of cercariae per snail for the period from 11:00 A.M. to 3:00 P.M. for this series of runs is 874 which falls well within the range of the overall series of averages presented in Table 3. However, if to this is added the production of cercariae for the mornings of the same day, then the average daily production of cercariae for a five and a half hour stimulation period comes to 1430 or double the overall average of Table 3. In Table 5 the dates on which these longer periods of stimulation were run are included so the figures can be related to the average values of Table 3 for the same period.

The Half Life Time of Infected Snails. In order to plan work ahead it is clearly important to know how long snails, infected by the standard procedure described,

and stimulated four or five times a week to liberate cercariae, are likely to survive. In Table 6 are collected data on several hundred snails all of which had been exposed to infection with the standard number (five to seven) of miracidia and all of which had begun to shed cercariae. The number of snails surviving at successive two-week intervals after each group had begun to shed cercariae are listed in the successive columns of this table. The time after the first liberation of cercariae at which half the snails have died is four weeks. This is the half productive life of the infected snails. It is very likely that the frequent stimulation to produce cercariae

TABLE 5.—*Average production of cercariae per snail during morning and noon hours*

Date	Number of snails used	Average number of cercariae produced per snail	
		9 : 30 A.M.–11 : 00 A.M.	11 : 00 A.M.–3 : 00 P.M.
Aug. 29 (1945)	16	281	343
Sept. 4	16	250	335
Sept. 11	18	488	319
Oct. 5	24	593	1803
Oct. 9	29	1078	455
Oct. 11	29	1727	110
Oct. 15	29	1041	1613
Oct. 17	28	367	1421
Oct. 22	29	2786	267
Oct. 24	29	17	144
Oct. 29	28	1960	1446
Oct. 31	27	430	425
Nov. 5	26	1580	1153
Nov. 7	29	33	124
Nov. 12	13	646	247
Nov. 14	12	900	515
Nov. 19	12	2308	450
Nov. 28	12	25	775
Dec. 3	12	100	1708
Dec. 5	14	17	232
Dec. 10	13	2215	392
Dec. 12	12	87	970
Dec. 17	19	14	1278
Jan. 2 (1946)	14	227	2224
Jan. 9	17	156	403
Jan. 14	17	520	284
Jan. 16	17	37	568
Jan. 21	17	222	640
Jan. 28	20	204	2472
Jan. 29	20	202	722
Feb. 4	19	223	1444
Feb. 11	19	0	1401
Feb. 18	16	400	2165
Feb. 25	26	259	913
Feb. 26	31	326	312
Mar. 4	31	276	1006
Mar. 7	34	749	596
Mar. 11	33	612	1182
Mar. 18	36	173	816
Mar. 22	49	23	1152
Mar. 25	47	38	1131
Mar. 29	46	302	554
Apr. 1	46	67	1034
Apr. 3	42	617	534
Average		558	874
Average per hour		372	218

shortens the life span of these infected snails. Manson-Bahr and Fairley (1920) pointed out that even under ordinary laboratory conditions infected snails did not live as long as normal ones even when great care was taken regarding feeding and supply of fresh water.

Half Life Time of Cercariae. Some exact information on the length of life of cercariae under the laboratory conditions of their production, collection and use for infection of mice seemed necessary for better control of these infections. The few studies that were made are summarized in Table 7. Three separate suspensions of cercariae numbered 1, 2 and 3 were prepared on three separate days diluting the cercarial suspensions liberated from the infected snails with Great Bear spring water

TABLE 6.—*Survival time of infected snails*

Number of snails producing cercariae	Number of survivors in weeks after initial production of cercariae							
	2	4	6	8	10	12	14	16
16	16	16	16	5	5	4	1	0
8	8	7	7	7	7	6	1	0
5	5	5	5	5	5	1	0	0
10	10	10	10	10	5	2	1	0
5	5	5	5	5	5	3	1	0
39	12	8	5	4	3	0	0	0
66	38	24	17	10	3	1	0	0
60	39	17	10	6	0	0	0	0
52	33	15	4	0	0	0	0	0
17	4	0	0	0	0	0	0	0
9	3	0	0	0	0	0	0	0
60	42	33	22	19	13	11	7	5
34	30	25	20	14	12	10	8	4
9	8	8	5	4	3	1	0	0
47	38	33	23	14	9	7	6	5
44	39	29	21	10	6	4	3	3
27	22	16	5	2	2	1	1	1
25	12	2	1	0	0	0	0	0
33	20	6	1	1	0	0	0	0
27	12	5	5	3	2	2	2	2
38	32	25	24	17	17	9	6	4
55	48	44	41	25	18	10	4	2
Totals 686	476	333	247	161	115	72	41	24

to give about 150 cercariae per ml. Each of these suspensions was divided into two parts and kept in a covered beaker in an incubator at 30° C. Counts on these cercariae were made at intervals and the results recorded in Table 7. The time the cercarial suspensions were first set in the incubator was arranged to be not more than three hours after the snails were first set under the lamp to stimulate cercarial production. Zero time was taken at about an hour after the snails were first set out. The half life of the cercariae under these conditions is between 8 and 16 hours

TABLE 7.—*Surviving cercariae per ml in suspensions kept at 30° C in several media*

Time, hrs.	Surviving cercariae per ml					
	Suspension 1		Suspension 2		Suspension 3	
			In Spring Water			
2	124	120	124	168	156	142
4	108	154	124	128	162	102
6	118	92	168	144	...	...
8	20	38	134	162	...	...
16	...	...	...	...	2	0
18	0	0	4	0	0	4
20	0	0	0	0	0	0
			*In Tyrode Solution**			
2	140	166	108	110	130	102
4	116	156	88	102	122	118
6	102	146	124	116	...	...
8	74	120	122	136	...	...
16	...	...	...	...	64	52
18	12	10	12	108	94	68
20	8	30	58	54	46	38
22	4	6	30	28	46	16
24	...	...	10	6	16	2
26	...	...	8	8	0	0
			In Tyrode Solution with .1% glucose*			
2	120	140	146	110	118	162
4	88	118	108	88	152	182
6	86	126	90	124	...	...
8	80	106	130	138	...	...
16	...	...	...	...	84	156
18	42	34	64	110	100	100
20	64	64	48	50	94	138
22	2	20	54	38	80	118
24	...	...	26	44	50	110
26	...	...	14	12	38	56

* A solution was made up with 0.8 gm. NaCl; 0.02 gm. KCl; 0.02 gm. $CaCl_2 \cdot 2H_2O$; 0.01 gm. $MgCl_2 \cdot 6H_2O$; 0.005 gm. NaH_2PO_4 and 0.1 gm. $NaHCO_3$ dissolved in 100 ml. distilled water; equal volumes of this solution and of cercarial suspension were mixed.

and no cercariae survived beyond 18 hours. During this time the pH of the suspensions did not change significantly, at the start it averaged 7.4, after 24 hours it averaged 7.3. For comparison some runs were made in a Tyrode solution instead of in spring water and a marked increase in half life became apparent. In this same Tyrode solution containing in addition 0.1 percent glucose the half life of the cercariae extended to about 20 hours with considerable numbers surviving 26 hours. Faust and Hoffman (1934) reported that 90 percent of cercariae died within 30 hours after emission from snails. Krakower (1940) gave survival times in rain water at room temperature or at 32° C as 24 to 48 hours with occasional survivors at 72 hours. Kuntz and Stirewalt (1946) found the time required for the death of all cercariae to range from 40 to 72 hours. Under the conditions of our experiments cercariae in spring water were never found to survive longer than 24 hours.

SUMMARY

Data on experimental infections of the snail *Australorbis glabratus* with the trematode *Schistosoma mansoni* are presented. The figures given are derived from experiments sufficiently extensive to have statistical validity. This validity applies only to the specific laboratory conditions described and the figures are not applicable to field conditions and natural infections. The figures presented are directed to laboratory workers concerned with chemotherapy of experimental schistosomiasis. The conclusions drawn from the data presented are as follows:

1. By the method described, exposure of snails to 1 miracidium per snail leads to infection of 8 percent of snails; exposure to 5 to 7 miracidia yields infection in 50 to 60 percent of the snails.

2. The time at which half of a given population of miracidia died is about eight hours.

3. Snails that become infected as a result of exposure to 5–7 miracidia liberate on the average 700 cercariae per snail per day when stimulated under the conditions described. On Mondays, after two days of rest from stimulation, this average is 1200. Illumination of snails at the time of stimulation of cercarial emergence by warming increases the average emergence by amounts from 30 to 90 percent.

4. The cercaria-producing half life of snails infected by exposure to 5 to 7 miracidia and stimulated to liberate cercariae 4 or 5 times a week is 4 weeks.

5. Half of populations of cercariae in suspensions of 150 per ml incubated at 30° C are dead between 8 and 16 hours after emergence from the snails; all are dead 20 hours after emergence. In Tyrode solution containing 0.1 percent glucose the time at which half such cercarial populations die is extended to 20 hours.

BIBLIOGRAPHY

BRACKETT, S. 1940 Studies on schistosome dermatitis. VI. Notes on the behavior of schistosome cercariae. Am. J. Hyg. **31D:** 64–73.

FAUST, E. C. AND HOFFMANN, W. A. 1934 Studies on schistosomiasis mansoni in Puerto Rico. III. Biological studies. 1. The extra mammalian phases of the life cycle. Puerto Rico J. Pub. Health and Trop. Med. **10:** 1–47.

HUNTER, G. W., BENNETT, H. J., INGALLS, J. W. AND GREENE, E. 1947 The molluscan intermediate host and schistosomiasis japonica. III. Experimental infection of *Oncomelania quadrasi*, the molluscan intermediate host of *Schistosoma japonicum*. Am. J. Trop. Med. **27:** 597–602.

Isobe, M. 1923 Biological observations on cercariae of the Japanese blood fluke. Taiwan Igakkai Zasshi (J. Med. Assoc. Formosa) March, No. 227. (In Japanese.) Trop. Dis. Bull. **20:** 943.

Krakower, C. A. 1940 Some observations of the effects of physical and chemical agents on the cercariae of *Schistosoma mansoni.* Puerto Rico J. Pub. Health and Trop. Med. **16:** 26–44.

Kuntz, R. E. and Stirewalt, M. A. 1946 Effects of DDT on cercariae of *Schistosoma mansoni.* J. Parasit. **32:** 529–538.

Maldonado, J. F. and Acosta-Matienzo, J. 1947 Longevity and rates of infectivity of the free swimming miracidia of *Schistosoma mansoni.* J. Parasit. **33;** Suppl.: 24.

Manson-Bahr, P. and Fairley, N. A. 1920 Observations on Bilharziasis amongst the Egyptian Expeditionary Force. Parasitol. **12:** 33–71.

Rees, G. 1931 Some observations and experiments on the biology of larval trematodes. Parasitol. **23:** 428–440.

Schubert, M. 1948 Conditions for drug testing in experimental schistosomiasis mansoni in mice. Am. J. Trop. Med. **28:** 121–138.

THE PROBABLE EXPLANATION FOR THE DIFFERENCE IN THE LOCALIZATION OF ADULT *TRICHINELLA SPIRALIS* IN YOUNG AND OLD MICE *

JOHN E. LARSH, JR. and JAMES R. HENDRICKS

Although in many textbooks it is stated that adults of *Trichinella spiralis* are found in the upper portion of the small intestine (Craig and Faust, 1945, and others), only two references were found dealing specifically with the localization of the worms in experimental animals. In neither case is the age of the animals given. Roth (1938) using guinea pigs found most of the adult worms in the lower portion of the small intestine and the upper portion of the large intestine. Tyzzer and Honeij (1916) found essentially this same distribution in rats and mice. Observations in this laboratory suggested that the age of the host may be in some way involved in the localization of the worms. It had been noticed repeatedly that in rats about six months old, the majority of the adult worms were found in the first half of the small intestine. The reverse of this found in rats about one month old suggested an interesting relationship worthy of further study.

Using mice as the experimental host a comparative study was made of the localization of adult *T. spiralis* in the intestinal tract of young and old animals. This was followed by an attempt to determine the mechanism involved in this localization. Most of the experimental procedures have been described in detail in an earlier paper (Larsh and Kent, in press), the remainder will be given below in the discussion of experiments.

EXPERIMENTAL RESULTS AND DISCUSSION

I. *The distribution of adult* Trichinella spiralis *in the intestinal tract of young and old mice*

A total of six experiments was performed using identical experimental procedures. This repetition was necessary because of technical difficulties which limited the numbers of animals that could be handled in one experiment. Young mice (28–32 days old) were matched according to sex with mice 130–140 days old, to be designated hereafter as "old" mice. Each mouse was given 300 *Trichinella* larvae by mouth, and, to cancel slight variation in dosage, mice from the two groups were infected alternately. Five days later the animals were sacrificed. The entire small intestine was removed and holding it under approximate uniform tension, it was measured to the nearest centimeter and cut into halves designated as A (the anterior half) and B (the posterior half). Each half was placed in a separate glass funnel and handled as in previous work (Larsh and Kent, *l.c.*). The following day counts were made of the adult worms found in each half (A and B) of the small intestine. The results of all six experiments are given in table 1.

It will be noted that similar results were obtained in all six experiments. In young mice the average percentage of adult worms found in the first half (A) of the small intestine varied from 5.5 in experiment four to 44.6 in experiment one. Thus

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the variation in percentage of adult worms found in the second half (B) of the small intestine was within the same range, from 55.4 in experiment one to 94.5 in experiment four. An average of all six experiments shows that, of the total adult worms recovered from young mice, 30.5 per cent were found in the anterior half (A) of the small intestine and the remaining 69.5 per cent in the posterior half (B). On the other hand, in old mice the average percentage recovery of worms from the anterior half (A) of the small intestine varied from 66.9 per cent in experiment four to 92.6 per cent in experiment five. Thus, the range in percentage recovery from the posterior half (B) was from 7.4 in experiment five to 33.1 in experiment four. Averaging all six experiments shows that, of the total adult worms recovered from the old mice, 81.3 per cent were from the anterior half (A) of the small intestine, while only 18.7 per cent were found in the posterior half (B).

These results showed, therefore, a striking difference between young and old

TABLE 1.—*Showing the distribution of adult* T. spiralis *in the small intestine of young and old mice. First half of intestine designated as A, second half as B*

Exper. no.	No. mice	Ave. Percentage Recovery			Ave. Percentage Location	
		A	B	Total	A	B
		I. Young mice				
1	8	17.0	18.6	35.6	44.6	55.4
2	8	9.9	16.3	26.2	42.7	57.3
3	7	2.9	17.1	20.0	17.4	82.6
4	6	0.7	15.1	15.8	5.5	94.5
5	4	12.6	23.0	35.6	35.5	64.5
6	4	11.8	18.1	29.9	37.1	62.9
Average		9.2	18.0	27.2	30.5	69.5
		II. Old mice				
1	7	21.7	9.1	30.8	71.3	28.7
2	5	14.2	3.3	17.5	84.5	15.5
3	6	21.7	7.0	28.7	81.5	18.5
4	3	3.7	2.0	5.7	66.9	33.1
5	5	34.9	2.9	37.8	92.6	7.4
6	5	32.1	3.2	35.3	91.1	8.9
Average		21.4	4.5	25.9	81.3	18.7

mice in the localization of adult *T. spiralis* within the small intestine five days post-infection. That such a difference was of statistical significance was shown by calculating the ratio of the observed difference to its standard error. This was done for experiment one in which the observed difference in localization of the worms was least in mice of the two age groups. On analysis, the observed difference was found to be 3.87 times the standard deviation of the difference. Since values greater than 2.00 are usually considered significant the results obtained were very unlikely to have occurred by chance alone.

It is also worth noting from table 1 that there is no evidence that the old mice were more resistant to infection than the young mice (average development of 25.9 and 27.2 per cent, respectively). This is in agreement with findings of Rappaport (1943) for three strains of *T. spiralis* in which no variation in age resistance to the parasite could be demonstrated in mice.

II. *The effect of intestinal emptying time on the distribution of* Trichinella spiralis *in the intestine of young mice*

The above results showed rather clearly that a much greater percentage of adult *Trichinella* localize in the posterior half of the small intestine in young mice than in old mice. The present series of experiments was performed in an attempt to determine the mechanism involved in this difference in distribution. Since mice of different ages vary in their physiological make-up, there are many factors that could be involved. However, the first of these to be considered is the rate of intestinal emptying time. In young mice this has been shown to be extremely rapid (Larsh, 1947), whereas in old mice the rate is much slower (Larsh, unpublished data). This suggested a possible explanation for the difference in localization of the worms. In testing this hypothesis one could artificially increase the intestinal emptying time in old mice and compare the localization of adult worms with that in controls of the same age given a similar test infection. However, because of greater availability of young animals it was decided to use them to test the hypothesis by artificially decreasing their intestinal emptying time.

One per cent morphine sulphate was selected for slowing the intestinal motility of the young mice (Larsh, 1947). Because the effects of this drug are so variable, and because of the use of a different parasite, it was necessary to perform the following preliminary experiments to determine the optimum time for drug administration which would allow for larval attachment.

Young mice, 30 days old, were divided into five groups. One of these served to test in non-drugged mice the rate of passage of carbon ink (Larsh, 1947) through the intestinal tract. Thirty minutes after receiving 0.1 cc of the ink, its presence was noted in the lower colon of these five controls. Each of the mice of the remaining groups received a 0.1 cc intraperitoneal injection of the drug prior to the ink. The drug was demonstrated to be active in all cases as shown by tail erection a few minutes after inoculation, a delicate test for morphine (Sollmann and Hanzlik, 1939). At 15, 30, 45, and 60 minute intervals after the drug was administered the mice of groups one, two, three, and four, respectively, were given 0.1 cc of the carbon ink by mouth. One of the mice from each of these groups was sacrificed 15, 30, 45, and 60 minutes after receiving the ink, and the stomach and entire intestine were removed. Because of the color of the ink it was possible to observe at a glance the distance it had traveled. The four mice sacrificed 15 minutes after receiving the ink all showed that none of it had escaped the stomach. With little variation, the mice of the four groups sacrificed after 30 minutes showed the ink within the anterior one-fourth of the small intestine. Likewise, those sacrificed after 45 minutes showed that the ink was confined to a distance somewhat less than one-half the length of the small intestine. In all mice examined within one hour after receiving the ink, some of it was found to remain in the stomach and in no case had it traveled beyond the first half of the small intestine. In a few additional animals sacrificed beyond one hour there was no ink in the stomach but it had traversed the small intestine and was found in large amounts in the caecum.

These results showed that drug action was operating for a maximum period of two hours. It was decided to give the drug one hour prior to infection. Since its action would continue for an additional hour, it would allow for larval attachment (Taliaferro, 1940; Gould, 1945). It is obvious that for other drug concentrations, this time interval would not apply but would need to be established by similar studies.

It may be seen from the above indirect approach that morphine sulphate, in the concentration used, has a pronounced slowing effect upon the passage of carbon ink. However, this was not proof that the same obtains for *Trichinella* larvae. It seemed advisable, therefore, to use a direct approach in determining this by substituting for the ink known numbers of larvae. In this case, the animals were all killed at the same time after infection and the distribution of worms noted.

Twelve young mice, 30 days old, were given the drug as described above and, after an interval of one hour, were each given 300 decapsulated larvae. Six non-drugged animals served as controls in which physiological saline was substituted for the morphine. These were given the same number of larvae at infection. Five days later the mice were autopsied and counts made of the number of worms in the first and second halves of the small intestine. Ninety-one per cent of the total worms remaining in the small intestine were in the first half in the drugged animals

TABLE 2.—*Showing the distribution of adult* T. spiralis *in the small intestine of young mice given morphine sulphate and in non-drugged controls. First half of intestine designated as A, second half as B*

Exper. no.	No. mice	Ave. Percentage Recovery			Ave. Percentage Location	
		A	B	Total	A	B
		I. Young mice given morphine sulphate				
1	4	17.7	2.1	19.8	90.5	9.5
2	6	65.4	5.4	70.8	93.2	6.8
3	5	84.0	1.0	85.0	98.8	1.2
4	8	54.5	2.1	56.6	95.6	4.4
Average		55.4	2.7	58.1	94.5	5.5
		II. Young mice given saline				
1	5	5.9	11.9	17.8	32.2	67.8
2	6	10.4	54.9	65.3	18.1	81.9
3	4	22.6	51.8	74.4	30.1	69.9
4	8	18.4	29.4	47.8	39.3	60.7
Average		14.3	37.0	51.3	29.9	70.1

as compared with 30 per cent in the non-drugged controls. It was, therefore, evident that the drug, as used, had the same general effect on retarding the passage of the larvae as of the carbon ink particles. The fact that there were three times as many adult worms in the first half of the intestine of drugged animals as of non-drugged controls indicated clearly that intestinal emptying time is involved in some way in this distribution. To substantiate this conclusion, however, it was necessary to perform additional experiments.

In all, four experiments of the same kind were carried out. The experimental and control mice were matched according to age (38–40 days), sex, and approximate weight. Food was then withheld from all the animals for a period of one hour. Thereafter, the experimental mice were given morphine sulphate as above, and the controls were given a similar amount of physiological saline. One hour later, alternating between experimentals and controls, all the mice were infected with 300 larvae. Five days later the animals were sacrificed and counts were recorded of the adult worms found in each half of the small intestine. These results are tabulated in table 2.

It will be noted from the table that similar results were obtained in each of the

four experiments. In the young mice given morphine sulphate, variation in the percentage of adult worms in the anterior half (A) was from 90.5 in experiment one to 98.8 in experiment three, while the percentage in the posterior half (B) ranged from 1.2 to 9.5. This is in striking contrast to the location of adult worms in the young mice given saline. In them the variation in percentage of adult worms in the anterior half (A) was from 18.1 to 39.3 and in the posterior half (B) from 60.7 to 81.9. It is worth noting, also, that the average percentage location of the worms, 29.9 in the anterior half (A) and 70.1 in the posterior half (B), corresponds favorably with the results obtained for the young mice of the previous study (table 1).

The striking difference in location of adult worms in the drugged and non-drugged mice leaves little doubt that the rate of intestinal emptying time is an important factor in the distribution. Direct comparisons were not made, but the results in young mice would seem to suggest that the striking difference in localization of the worms in young and old mice (table 1) was due chiefly to the difference in intestinal emptying time.

The fact that so few adult worms (average 5.5 per cent) were found in the posterior half of the small intestine of the drugged mice suggested the possibility of limiting all of them to the anterior one-half by prolonging drug action. One additional experiment was performed to test this hypothesis. Twenty young mice (35 days old) were divided into three groups. Two of these groups were treated the same as in the above study. Four mice in one group served as non-drugged controls given saline one hour prior to infection with 300 larvae. Eight mice in the second group were given one injection of the drug as above one hour prior to the infection. The eight remaining animals were used in an effort to prolong the duration of the drug's action. These were given an initial injection of the drug one hour prior to infection and additional injections at six hour intervals for 48 hours. Since the drugged animals showed no interest in food it was necessary to give each of them, at six hour intervals during the time of drug action, a 0.5 cc intraperitoneal injection of five per cent glucose in physiological saline. Soon after this period they began eating the stock food. All the animals were autopsied five days after infection and the percentage location of the adult worms determined.

The non-drugged controls and the drugged animals given only one injection of morphine showed about the same distribution as above (table 2). The former showed 35 per cent of the adult worms in the anterior one-half and 65 per cent in the posterior one-half, while the latter showed about the reverse (90 and 10 per cent, respectively). There was very little difference between these last figures and those for the mice maintained on the drug constantly for 48 hours (92 per cent in the anterior one-half, and 8 per cent in the posterior one-half). This would seem to indicate, therefore, that the worms found in the posterior portion of the intestine of drugged mice (table 2) was not due to insufficient duration of drug action.

SUMMARY

Studies are described showing a distinct difference in the localization of adult *Trichinella spiralis* in young and old mice. In young mice significantly greater numbers were found in the posterior one-half of the small intestine (average: 69.5

per cent), while the reverse was true in old mice (average: 18.7 per cent). Therefore, age apparently is a factor involved in this localization. Approximately the same percentage development of adult worms was found in mice of both age groups, showing no evidence of age resistance.

In an attempt to demonstrate the mechanism of this difference in distribution, a study was made of the role of intestinal emptying time which is known to differ considerably in young and old mice. A reversal in distribution was shown in young mice by artificially decreasing their intestinal emptying time with morphine sulphate. In this case approximately 95 per cent of the worms were located in the anterior one-half of the small intestine. Although a direct comparison with old mice was not made, these results would seem to indicate that the difference in localization of the adult worms in mice of the two age groups is due chiefly to the difference in intestinal emptying time.

References

Craig, C. F. and Faust, E. C. 1945 Clinical Parasitology. Lea and Febiger, Philadelphia. 871 pp.

Gould, S. E. 1945 Trichinosis. Charles C. Thomas Co., Springfield, Illinois. 356 pp.

Larsh, J. E., Jr. 1947 The relationship in mice of intestinal emptying time and natural resistance to *Hymenolepis*. J. Parasitol. **33**: 79–84.

———Unpublished data.

Larsh, J. E., Jr. and Kent, D. E. The effect of alcohol on natural and acquired immunity of mice to infection with *Trichinella spiralis*. J. Parasitol. (In Press).

Rappaport, I. 1943 A comparison of three strains of *Trichinella spiralis*. I. Pathogenicity and extent of larval development in the musculature. Am. J. Trop. Med. **23**: 343–350.

Roth, H. 1938 On the localization of adult *Trichinella spiralis* in the intestinal tract. J. Parasitol. **24**: 225–231.

Sollmann, T. H. and Hanzlik, P. J. 1939 Fundamentals of Experimental Pharmacology. J. W. Stacey, Inc., San Francisco. 307 pp.

Taliaferro, W. H. 1940 The mechanism of acquired immunity in infections with parasitic worms. Physiol. Rev. **20**: 469–492.

Tyzzer, E. E. and Honeij, J. A. 1916 The effects of radiation on the development of *Trichinella spiralis*. J. Parasitol. **3**: 43–56.

ANNOUNCEMENT

On December 2, 1948, fire destroyed the School of Medicine of the University of Santiago, Chile, including the library, all equipment and preparations of the Department of Parasitology. Members of the American Society of Parasitologists are requested to send reprints and microscopic preparations to Professor Amador Neghme, P.O. Box 9183, Santiago, Chile.

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No. 5, November 16.
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